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A Consideration of Ensiled, High Moisture Barley

and its Utilization by Ruminants.

Stephen B. J. Dunne. B.Sc. (Honours).

University of Leeds.

A Thesis Submitted for the Degree of Master of Science

in the

Faculty of Science

of the

University of Glasgow.

Department of Nutrition and Microbiology,

The Scottish Agricultural College,

Auchincruive,

Ayr.

September 1990.

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A paper relating to this research study was presented by
the author at the

Ninth Silage Conference,

Faculty of Agriculture,

University of Newcastle upon Tyne.

September, 1990.

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Abbreviations.

<i>ad libitum</i>	Freely.
°C	Degrees Centigrade.
cm	Centimetre.
d	Day.
DM	Dry matter.
<i>et al.</i>	and others.
FW	Fresh weight.
g	Grams.
HDMI	Hay dry matter intake.
hr	Hour.
kg	Kilogram.
LW	Live weight.
m	Metre.
meq	Milli-equivalent.
mg	Milligram.
ml	Millilitre.
mm	Millimetre.
OM	Organic matter.
OMD	Organic matter digestibility.
P	Probability.
PFOM	Protein-free organic matter.
SED	Standard error of difference.
t	Tonne.
μm	Micrometre.
μl	Microlitre.
VFA	Volatile fatty acids.
vs	Versus.
<	Less than.
*	Significance level.

Acknowledgements.

I wish to acknowledge the advice and guidance given by Dr. N. W. Offer for the completion of this study.

I also wish to thank Mr. J. Weir for his practical assistance.

The help given by the personnel of the Scottish Agricultural College, Auchincruive, who offered a practical contribution to this research is appreciated.

This M.Sc. was supported by:

The Ministry of Agriculture, Fisheries and Food.

S.B.J.D.

Nothing is more wanting in agriculture than experiments in which all the circumstances are minutely known and scientifically detailed.

Sir Humphrey Davy, 1821.

To raise new questions, new possibilities, to regard old problems from a new angle requires creative imagination and makes real advances in science.

Albert Einstein, 1941.

ABSTRACT.

Barley is physiologically mature at 30% to 40% moisture; 3 to 4 weeks before traditional harvest time. Acid/ formaldehyde additives have been found to reduce rumen degradation of the grain protein and starch, giving a number of advantages in animal production. The effects of a range of additives on the preservation, rumen degradability and feeding value of High Moisture Barley (HMB) were investigated in three experiments:

1. HMB (Atem variety), harvested at 451 g/kg moisture was crimped (bruised, cracked or kibbled) and ensiled without additive or simultaneously treated with one of three additives at various application rates. The treatments and application rates used were: 'Graintona Plus' (8 l/t & 4 l/t), 'Sylade 2' (8 l/t & 4 l/t) and Cane Molasses (6.4 l/t). Each of these treatments was ensiled in triplicate 4 kg laboratory pot silos and five small (0.3 kg) pH pots. Duplicate 150 kg silos were used for all ensilations apart from the untreated grain. The untreated grain, the majority of which was not ensiled, was frozen until required. Treatments involving molasses, 'Forager' Inoculant, 'Add-Safe' and amylase were prepared from the untreated grain and ensiled in triplicate 4 kg pot silos only.

Graintona and Sylade reduced fermentation in the silo, producing low levels of lactic and acetic acids, together with the highest pH values. Unlike the other additive treatments, they allowed a greater mould development. Sylade, together with the molasses treatments, gave high ethanol contents.

2. Graintona, Sylade and molasses were used for *in sacco* degradability studies. The rumen degradability of barley

nitrogen, organic matter and protein-free organic matter (mostly starch), was measured using the nylon bag technique in three mature, rumen fistulated sheep.

Graintona and Sylade, used at 8 l/t, significantly reduced ($P < 0.05$) the degradability of barley nitrogen, organic matter and protein-free organic matter. The instantaneous loss from the bags was most affected and the reduction diminished with increasing incubation time.

3. A feeding trial of incomplete block design, using ten individually penned yearling sheep, was used to determine the effect on hay intake and diet digestibility of formaldehyde-treated grain. The trial involved three 20-day periods. During days 1-10, the sheep (average live weight 34.5 kg) received hay *ad libitum* and 700 g/day (fresh weight) of HMB. Both treatment levels of Graintona and Sylade were used for the individual meals, together with molasses or thawed, untreated HMB. The six meals were distributed according to the experimental design and fed once per day at 08:45. A mineral supplement of 15 g/day was added to each meal. Hay and water were always available. During days 10-20, hay was restricted to 500 g/day (fresh weight) and a total faecal collection was made during the last three days.

The HMB meals were consumed rapidly, in all cases within one hour. The sheep remained healthy throughout the trial, no incidence of acidosis occurred and each one made live weight gains. Under the conditions used in this experiment, there was no significant effect of treatment on hay intake or whole diet organic matter digestibility. It is suggested that with moderate barley intakes, rumen conditions on the control diet cannot have changed significantly from normal and in this growing, fattening

trial, an effect of formaldehyde protection of barley nitrogen and protein-free organic matter on hay intake was not observed.

The overall results of the experiments suggest that HMB can be ensiled successfully and can be used as a valuable source of 'home-grown' feed. Modifications of the rumen environment by formaldehyde treatment of barley would be most beneficial for animals in commercial, high production systems.

PART ONE.

REVIEW OF THE LITERATURE.

INTRODUCTION.

The greatest quantity of barley cereal grain fed to ruminants is given as a supplement to diets based on fibrous roughage. The aim being to increase the overall intake of digestible energy in order to achieve the high output levels demanded by modern intensive production systems. Yet the complex digestive system of the ruminant has evolved to allow efficient digestion of fibrous feeds. Therefore, the feeding of diets containing high proportions of cereals represents an unnatural development.

Research conducted by scientists in a number of countries, especially the USA, Canada, the UK, Australia and Scandinavia, has suggested that ensiled high moisture grain; especially barley because of its unique characteristics and wide ecological adaptability, is an effective nutritive for the production and health of domestic ruminants. The scientific studies which have taken place on the subject have shown a number of interesting factors, but the data obtained in some studies has been variable, inconsistent and, in some cases, contradictory. As Grovum (1985) stated -

'further research.... is needed to establish a good foundation for modern production practices as well as for the treatment of disease.'

It is of paramount importance to know the extent to which barley cereal grain may be utilized in animal diets, especially as future trends underline the need for greater efficiency in animal production. Research success is necessary in order to use all resources wisely to meet the demands of both future human and animal populations. The requirements for human food are increasing more rapidly than world output of cereals and protein, therefore less is available for the animals.

This research study will comprise of three linked component parts and each one will centre on a specific experimental area in relation to High Moisture Barley and the ruminant. The first will encompass the ensilation process and the properties of the Atem variety of barley. The second will involve *in sacco* experimental investigations and the third will comprise of a feeding trial with young sheep. It is hoped that this work will provide some valuable information on the ensilation process and the feeding value of High Moisture Barley for ruminants and also that it will furnish some additional knowledge to all those who have an interest in this field of study.

1.1 HISTORICAL.

Historically, the concept of crop storage has been one of the most important discoveries made by mankind to ensure his continued existence.

Initially, the stores formed a substantial food reserve from one season to the next, or in periods of cold weather, drought or famine. As time progressed, these stores, especially those containing seeds and roots, enabled a pattern of domestic agriculture to evolve. Within all cultural civilisations, the most important crops chosen to be stored have always been the cereal crops, of which barley is an important member.

It is through the process of storage, that it has become known, that barley is as ancient as the origins of agriculture itself and that it was used by the ancient civilizations. The earliest reports on the domestication of barley, especially the two-rowed variety, come from many archaeological studies and findings. The antiquity of barley is documented from grain, husks and grain fragments unearthed at sites in the Near and Middle East. Barley carbon dated to the historical period 7000 BC, or probably earlier, has been discovered. Finds in other locations, dating historically to an even earlier period in time than this, have also been presented by other archaeologists. Biblical references illustrate the importance of barley as a food source in Old Testament times.

Along with these finds of cultivated barley are many of those which indicate that man's domestic animals, especially the Ruminantia group of sheep, cattle and goats, existed in this early pattern of subsistence agriculture. The uniqueness of ruminants, as compared to other animals, must have been recognized (probably even by the primitive hunters) long ago.

There is extensive literature on agriculture in Roman times. It is of interest to note that Roman gladiators were known as 'hordearii', because of their belief that barley provided a source of physical strength for man. In 1240, a large book relating to the growth of plants such as barley in agriculture was hand written by Petrus Crescentius, a senator from Bologna, Italy (Russell, 1988).

The grain and roots grown and obtained in Summer, which were stored by man, were predominantly designated for human consumption and not for animal food. The storage of food, especially Winter feed for animals commences from a much more recent historical period of time. Bread made from barley formed the staple diets of people, especially those living in the country, in the Middle Ages and the 15th Century in Britain, as it did throughout all the European countries. In the 15th and 16th Centuries, more literature on domestic agriculture appeared in Italy, which spread ideas and theories northwards to France and throughout the rest of Europe. Until the end of the 17th Century, the meat and dairy produce, which was consumed by the people in the Winter season, was practically confined to butter and cheese and to salted and dried meats, preserved during the Summer and Autumn seasons. Sheep which had become established (as a result of the enclosure acts) and pigs outwintered on rough grazing and in the forests. Extremely few cattle or dairy cows were fed during the Winter, only those required to feed the wealthy in the population.

Around the period 1793 to 1815, harvest failures occurred and there were severe shortages of food, especially barley. Barley was the main staple for bread in rural areas of Denmark and Europe, as well as Britain until the 20th Century (Horn,

1987). It was not until the period of the 1800's that the great demand for food, especially meat, from the populations in the new industrial cities made it necessary for farmers to feed and maintain animals during the Winter period. The basic need for food during the Industrial Revolution brought about the Agrarian Revolution. The feeding of these animals was not based on home grown conserved forage. Supplies of large quantities of grain from Europe and USA and oil cake from Russia and Africa were available for the farmers of Britain. This resulted in the establishment and development of a system based on a livestock cycle, in which animals grazed throughout the Summer months and were fed coarse fodder and concentrates during the Winter period. In 1885, Kornicke produced a valuable documented account on wild and cultivated barley, which made a great contribution to the new found interest in agriculture (Rasmusson, 1985).

Numerous methods of storage have been devised, some more successful than others. Complete successful storage is that which prevents deterioration. There are two ways in which deterioration may occur. The barley grain itself may deteriorate or, providing suitable conditions prevail, other living organisms may utilize the barley as a substrate. Cereal grains, of which barley is an important member, are living embryos and contain a food reserve (starch) in the endosperm. If a suitable temperature, sufficient moisture and oxygen prevail, germination will commence. Insects, mites, bacteria and moulds will also multiply in the grain store, utilizing the barley food reserve for their own nutrient supply. Both the grain and other living organisms present will, as they respire, constantly produce heat and moisture, continually improving the conditions for their growth and accelerating the process of deterioration. It is thought that each year,

approximately one third of all the harvested crops in the world may be lost due to inadequate storage.

A method of storage quite distinct from the traditional one of drying the grain is that of ensilation. This is a method by which a crop of grain containing a high moisture content is preserved. The concept of storing grain of a high moisture content for use later as a feed supply for animals is not a new phenomenon. Interest in the storing of high moisture grain by the method of "en silage" arose in France more than a century ago, in 1869. It was devised as a method to help solve the serious problems of provisioning the garrisons and of feeding the 2000 horses belonging to the General Omnibus Company of Paris (Muntz, 1881).

An alternative way of ensuring the maximum preservation of an ensiled crop is by the addition of chemical preservatives. The original concept for the use of acids as additions to ensiled stores dates back to 1885. The original objective in using the chemical additives was to ensure that the lactic acid bacteria dominated the fermentation process, resulting in maximum preservation. The earliest known use of lactic acid bacterial culture in ensilation was by workers in France, on sugar beet pulp. The role of bacteria in the fermentation of plant materials became well known as a result of the work of Pasteur in 1863.

In 1929 A.I. Virtanen, working in Finland, devised a different method to preserve an ensiled crop. He scientifically researched the details of the "en silage" method, which had originally evolved in France and recommended rapid acidification of the crop with mineral acids to a pH of approximately 3.5. His proposals were well accepted and the process, known as the A.I.V. process, was widely used in Scandinavian countries. The use of

formic acid as a silage additive had been advocated by Dirks in 1926 and a few years later, Von Kapff described a 'silo acid' process in which a mixture of formic acid and hydrochloric acid was sprinkled over the ensiled crop.

In France, between 1938 and 1939, Blanc tested the original methods of "en silage". Moderately damp grain of 162 g/kg moisture content was used in his experimental work. As a result of these tests, storage was developed commercially in France for grain of moderately high moisture content. The original acid produced for commercial use by Virtanen was composed of a mixture of hydrochloric and sulphuric acids. This was to be diluted with water prior to application. Later, other acids were included in the mixture and one product retailed in the 1930's included phosphoric acid instead of sulphuric. In the Penthesta process, phosphorus pentachloride was added to water to produce a mixture for ensilage (Raymond, 1978). Molasses was also used extensively. In the UK, processes using chemical preservatives proved to be unpopular, because of the constraints in handling corrosive acids and there was a definite wain of interest in their use.

Through the dedicated work of scientists, agronomists and researchers it has been known for many years that barley grain reaches its maximum production, or, its physiological maturity, several weeks before the traditional, dry harvesting period. In 1912, Brenchley researching at Rothamstead, UK, reported that the dry matter yield of barley reached a maximum three weeks before the traditional harvesting period. In the 1920's and 1930's, a number of scientific researchers renewed the interest in experimenting with grain of a high moisture content, especially barley, because of its unique qualities. In 1920, Harlan at Aberdeen, Idaho, researched the development of the barley kernel.

He found that no translocation of plant materials occurred after the moisture content lowered to 42%. This state was reached between nineteen and twenty one days after flowering. In 1923, he reported that the percentage of water decreased daily until the 42% moisture level was reached; thereafter, the barley kernel dried very rapidly at the rate of 2% each day. Koenig of Colorado reported from his research on barley (for malting purposes), that no increase occurred in kernel weight after the moisture content fell below 33%. In 1933, McLean reported from his research work in Canada that there was no significant reduction in either yield or kernel weight of barley, harvested seven days before maturity (Krall, 1969).

Also at this time, experiments using high moisture grain were conducted in the USA. On many of the early farms and ranches, in the USA and Canada, the feeding of moist grain to cattle, pigs and poultry was common practice. The moistening of grain was accomplished in a variety of ways. Grain soaked in water, or skimmed milk, was often fed to pigs. In many cases this may have been fermented grain. Soaked and cooked grain was often fed to pure bred and show cattle. Maize with a high moisture content was used in early experiments and despite some deterioration, the ensiled grain was fed successfully to pigs (Krall, 1969).

As a result of the growing demands of the livestock industry, numerous research studies concerning ruminant digestion of the three major cereal grains; barley, maize and sorghum, using crossbred steers had begun at a number of University Experimental Stations in the USA. Waldo had begun research at the University Department of Agriculture, Maryland; Van Soest at the Cornell; Hale at Tuscon, Arizona, using barley and milo with

steers and lambs and Rooney, Theurer, Spicer and colleagues at the University College Experimental Station, Arizona, Texas. These research studies included the effect of moisture, temperature and pressure on *in vitro* starch digestion, the effect of drying, steam processing and flaking barley and milo on their digestion by ruminants and on animal performance.

Interest in the potential of high moisture grain as a feed source continued to increase in Canada and scientists began to research in various fields of study. Between the years 1952 to 1957, Dodds and his colleagues in Canada completed experimental studies using two varieties of barley. The results from these experiments showed that there was no reduction in the yield of the crop harvested five to seven days earlier than the maturation time of the barley. In 1960, the actual use of combine harvesters to harvest barley with a 21% to 47% moisture content was reported by researchers at the Fargo Experimental Station, North Dakota. In 1961 and 1962, barley with a moisture content of between 23% and 35% was harvested with combine harvesters at Crookston Experimental Station, Minnesota. Also in 1962, five varieties of barley with a moisture content ranging from 18% to 48% were harvested at Huntley Agricultural Experimental Station, Montana, Canada. All resulting reports indicated that to direct combine barley at a high moisture content presented no difficulties and that it could be threshed quite easily. Successful feed trials were later conducted. Reports from research experiments using ensiled high moisture barley for fattening steers were presented by Fredrick and Reiner in 1962 and from Dinusson and colleagues in 1964 on the utilization of high energy barley in high energy beef rations.

Between the years 1963 to 1967, J.L. Krall working at the Huntley Agricultural Experimental Station in Montana, Canada began a series of intensive research experiments and studies growing, harvesting and ensiling barley of a high moisture content. The objectives of the experimental trials were in two research approaches and took place at five locations in Montana. Eight varieties of barley were used for the series of feed trials and investigations of harvesting and ensiling techniques. The varieties were: Dekap, Betzes, Ingrid, (two-rowed varieties); Unitan, Vantage (six-rowed varieties); Compana, Hypana and Lico X Ogalitsu.

By this time, important literature on formaldehyde had been produced by J.F. Walker in 1944. Literature on the chemistry of starch had been presented by Kerr also in 1944 and 1950 and on the structure of the mature corn kernel by Wolf *et al.* in 1952. In 1964, Whistler had provided literature on 'Methods in Carbohydrate Chemistry (Starch)' which was an excellent reference for the method of starch analysis.

Also in the 1960's, considerable research on the storing of moist grain was conducted in the UK because here, standing grain is subject to frequent rain and high humidity. In 1960, Hyde and Oxley presented a report on the research experiments they had conducted on the airtight storage of damp grain. They opened bins storing high moisture barley for the first time in March 1957, after 80 weeks successful storage. Their research on the internal processes such as gas exchanges, temperatures and microbiological activity presented a better understanding of strategic requirements for storage. In 1965, at Boxworth Experimental Husbandry Farm and the Gleadthorpe Experimental Husbandry Farm, the Agricultural Research Council devised new means of sealing

grain and invented the concrete staved silo for ensiling high moisture barley. Then in 1967 at Gleadthorpe, the Butyl, nylon reinforced, rubber bag was proved to be an excellent method of storage for this kind of barley, as it was more hard-wearing and gave greater protection from rodents and mildew than plastic. High moisture barley which had been successfully ensiled was fed to dairy steer calves (destined for slaughter) at Gleadthorpe. The reports from these experimental trials indicated that the high moisture barley was readily consumed by all the animals; it was easier to keep the cattle on feed with moist as compared to dry barley and there was no occurrence of digestive problems. In the UK, despite intensive research, chemical additives had little effect on practical ensilation until 1965, when Aas and Naerland (Norway) developed a simple applicator. In 1967, this efficient applicator was introduced to the UK and was used in trials at research stations, on husbandry farms and on selected livestock farms which confirmed the success of the Norwegian experience (Raymond, 1978). In 1969 it was released commercially. The availability of effective chemical additives and efficient applicators allowed the use of additives for ensiled crops to become, for the first time, a viable, practical method of storage. Also in 1969, research data and the use of acid-treated, ensiled high moisture barley in feed trials with beef cattle were initially reported by B.P. Chemicals, UK. In the 1960's, intensive research (which had commenced in the late 1950's) concerning ruminant digestion, carbohydrate metabolism and intestinal carbohydrate digestion in sheep was conducted by Armstrong, Beever and colleagues at the University of Newcastle. At the Rowett Research Institute, Aberdeen, Scotland, research by Orskov and Kay concerning the use of salts of volatile fatty

acids in lambs and post ruminal digestion of carbohydrates in sheep and lambs was in progress. Also, at the National Institute of Research, Reading, experiments concerning nitrogen metabolism in calves and the effects of formaldehyde and acid treatments on cereals were begun. In 1967, Krall presented the findings of his research at the 18th Annual Montana Nutrition Conference in Canada and a very great interest in the results was shown by other researchers involved in scientific studies related to the role of high moisture grain in the nutrition of ruminants. At this time there was a considerable amount of evidence to show that grain in the high moisture state could be well preserved through ensilation and it was well known that the physical processing of the grain improved utilization by ruminants; but there was limited information relating to the effect of grain containing a high percentage of moisture on animal growth, energy metabolism and nutrient utilization, especially with ruminants.

1.1.1 Research and Experimental Developments since the 1970's.

The publication of the excellent work by Krall revived interest and enthusiasm and stimulated research in many countries. At the beginning of the 1970's, there was renewed interest in ensiled high moisture barley as a replacement for dry barley (and also other cereal grains) in the diets of ruminants; especially in the use of chemical additives to protect plant protein and starch from microbial degradation in the rumen and on grains which had undergone varying degrees of processing. With the knowledge presented by these theories, further scientific research continued in the USA, Canada, Scandinavia and the UK and new work was begun in France, Japan, Australia, Israel and other countries.

Barley is cultivated as the predominant feed grain in Canada

and those areas of the USA where maize and sorghum are not adapted. The barley is used as pasture or hay but mostly the majority of crops are harvested for silage or grain. The early harvesting of cultivated barley in the high moisture state has proved to be of great advantage to the farmers on the central plains and in the intermountain valleys. In the USA, the majority of research studies which were conducted with cattle, especially crossbred steers and beef cattle, involved the acid treatment of the three major cereal grains: maize, barley and sorghum (Clark & Harshbarger, 1972; Forsyth et al., 1972; Flipot & Pelletier, 1980; Kennelly et al., 1988a,b). These studies indicated that acid treatment of high moisture grain is equivalent to dry, mature grain on a dry matter basis for body weight gains and they also suggested that it was superior in terms of feed efficiency. The responses varied, depending on the grain species, the moisture content, the concentration of acids, the amount of processing of the grain and/ or type of roughage fed and several animal factors. Throughout the 1970's, research was continued by Waldo and colleagues at the University of Maryland. In August 1972, a Symposium on 'Starch Utilization in Ruminants' was held at the 64th Meeting of the American Society of Animal Science, Blacksburg, Virginia and Waldo presented a comprehensive paper on his work. From 1971, Owens and colleagues at Oklahoma University conducted studies using formaldehyde-treated high moisture grains with cattle, sheep and lambs. At Arizona University, Texas, Rooney and Pflugfelder (1986) researched exclusively with varieties of sorghum, using chemical, physical and microscopic analysis. They reported that sorghum varieties differ widely in their processing properties and that sorghum starch is the most resistant to digestion in ruminants. They also stated that kernel

hardiness, size and rate of water uptake are among the most important factors affecting processing. Between the years 1970 and 1986, Spicer and Theurer, also at Arizona University, continued their work and completed numerous research studies on the ruminal and post-ruminal utilization of protein, starch and nitrogen with crossbred beef steers fed sorghum, maize and barley-based diets. They found that the amount of starch and source of amino acids available for post-ruminal digestion differed markedly among the three feed grains and also that, the apparent total tract crude protein digestibility of sorghum and barley were related to the extent of feed degradation in the rumen.

In Australia and New Zealand, interest was shown in ensiled high moisture grain, its treatment with acids and its effect on digestion and ruminant growth. Major studies were performed in New South Wales. Scientists working there added to their extensive research work of the 1950's and 1960's, when Hogan and Weston, also Hemsley, Ferguson and colleagues had investigated nutrition, wool growth and the protection of dietary protein from microbial degradation in the rumen. In the 1970's Faichney and fellow researchers conducted experiments using Leicester X Merino sheep, lambs and young calves. These included the digestion of concentrates by sheep and lambs; the effect of formaldehyde treatment on casein, peanut meal, barley and other concentrates in the diet of sheep, lambs and calves together with its effect on the growth of lambs. Changes in the composition and the pH of digesta along the gastro-intestinal tract of sheep were also studied. In 1974, the Fourth International Symposium on Ruminant Physiology was held in Sydney, Australia. Papers relating to 'Digestion and Metabolism in the Ruminant' were presented by many

leading research scientists; notably Armstrong (UK), Faichney (Australia), Ulyatt (New Zealand) and Van Soest (USA). At the University of Sydney, Lindsay and Leibholz (1983) investigated the digestibility of barley-based diets containing different amounts of formaldehyde-treated soya bean meal by Friesian calves.

Towards the mid 1980's a review on starch and the factors affecting it in food for humans was produced by Thorne *et al.* (1983) and a nutritional perspective on starch digestibility was presented by Dreher *et al.* (1984). These reviews provided excellent references and brought further knowledge to scientists and all those interested in this field of study. Also in 1984, the original work compiled by R. Whistler on 'Methods in Carbohydrate Chemistry' in 1964 was updated to 'Starch Chemistry and Technology' by the author and colleagues, to provide a comprehensive coverage on most of the information available on this subject. As a result of popular interest at this time, a considerable amount of literature relating to the subject of starch was published. Among those of special interest are 'Gelatinization of Starch and Mechanical Properties of Starch Pastes' by Zobel (1984) and 'Chemical Modification and Degradation of Starch' by Fleche (1985). Also in 1985, Kobayashi *et al.* published work on the 'Rapid Analysis of Starch, Amylose and Amylopectin by High Performance Size Exclusion Chromatography' and in 1986, 'Dietary Fat and Human Health' by Brisson was published, which underlined some of the very serious implications concerning the feeding of ruminant animals.

In August 1985, a Symposium entitled 'Starch Utilization in Ruminants' was held at the 77th Annual Meeting of the American Society of Animal Science, University of Georgia, Athens. Papers

were presented by the leading USA researchers: Rooney, Pflugfelder, Owens, Theurer and, from the Rowett Research Institute in the UK, E.R. Orskov; who through their work have made an outstanding contribution in this field of research. The findings of this symposium are considered in detail in the relevant sections of the literature.

Extensive research and investigation on all aspects of barley cereal has continued in Canada, using steers, cows in lactation, Holstein calves and sheep, since Krall published his intensive studies at the beginning of the 1960's. Some of the most interesting and important were those conducted in the 1970's by Ingalls, Clark and Sharma at the University of Manitoba; who researched nitrogen metabolism, digestion of acid-treated high moisture barley for lactating cows, digestion of formaldehyde-treated rapeseed meal and sites of digestion in sheep. Veira and colleagues investigated the feeding of high moisture maize preserved with propionic acid for beef cattle and cows and the nutrition of weaned Holstein calves (Owens et al., 1986). In the late 1980's at the University of Alberta, Mathison and colleagues researched the digestibility of high moisture grain treated with sulphuric acid. Kennelly, Mathison and fellow workers (1988 & 1989) conducted experiments into the relative feeding value of dry and high moisture barley using large numbers of feedlot cattle; together with the efficacy of anhydrous ammonia and sulphur dioxide as preservatives for high moisture grain, plus their effect on the nutritive value of barley for growing, finishing cattle.

Research which was first initiated over sixty years ago by Virtanen into ensiled high moisture grain has continued in Finland and Norway. In the 1970's, Larsen, Jorgensen and

colleagues researched the effect of organic acids on the preservation and acceptability of high moisture maize. In the 1980's, Setälä and Qvist studied *in vitro* degradation and utilization of formaldehyde-treated urea by rumen microbes (Ravelo et al., 1988). Today in Finland, it is now normal practice to grow mixed crops for high moisture ensiling. Not only is barley ensiled as a high moisture whole crop but it is also ensiled with peas, beans and oats as part of a mixed crop. The 'Murska' machine, with the unique roller for crimping moist grain previous to ensiling, used in this study, was designed and built by Gunner Körte, a farmer in Finland.

In the UK, experimental work has continued since the 1960's at all the major research centres. Armstrong and colleagues working at the University of Newcastle have researched extensively using sheep. Their work centred on carbohydrate metabolism in ruminants, the digestion and degradation of untreated/ formaldehyde-treated soya bean meal also; the absorption and metabolism of glucose in sheep fed cereal based diets. Research into carbohydrate digestion and glucose supply in the intestines of ruminants has been conducted at the National Institute of Research, Reading. At University College, Bangor, North Wales, N.W. Offer, Evans and Axford (1971) furthered research with the development of an automatic method of sampling digesta *in vivo*. They also investigated the protection of dietary protein by formaldehyde treatment and its effect on the composition of duodenal digesta in sheep. Morgan and colleagues from Dublin (1989), researched the effect of formaldehyde treatment of barley upon degradability and nutrient flow to the intestines of sheep. At the Rowett Research Institute, Aberdeen, Orskov, working with colleagues Kay, Fraser, McDonald and Mann,

continued his extensive research studies using cattle, sheep and lambs. Using both barley and maize cereal, they have studied dietary factors influencing the digestion of starch in the rumen, small and large intestines of early weaned lambs; the effects of processing cereals and barley-based supplements and the estimation of protein degradability. At the Hannah Research Centre, Ayr, experiments have continued with ruminants, especially lactating cows and sheep. Kassem *et al.* (1987) investigated silage intake and milk production in cows given barley supplements of reduced ruminal degradability. Van Ramshorst and Thomas (1988) studied digestion in sheep of diets containing barley chemically treated to reduce ruminal degradability also Martin and Thomas (1988), researched the effects of untreated or formaldehyde-treated barley and oats on milk fatty acid composition. At the West of Scotland College, Ayr, work has also centred on the potential of treated barley cereal for ruminant feed. J.J. Hyslop, N.W. Offer and colleagues (1989) have investigated the effect of formaldehyde on the rumen digestion of barley and wheat and its influence on hay intake and digestion by sheep. The findings of these recent studies will be considered in detail, both in the relevant sections of the literature and in the discussion.

1.1.2 Latest Research and Experimental Developments 1990.

The demands from the livestock industry account for the intensity of barley cultivation in the USSR, USA, Canada, Australia, New Zealand and NW Europe, including the UK. Today there is expanding world-wide research into the potential of ensiled High Moisture Barley, to establish it more positively as a valuable nutritional source for ruminant animals. Knowledge of the processes in the rumen after feeding grain has been gained

and recent studies have concentrated on microbial or metabolic changes along the gastro-intestinal tract of ruminants. As a nutritional source, cultivated barley has excellent potential. The development of chemical treatments of ensiled High Moisture Barley (and most other grains) has caused considerable interest, but more work is required. Today cereals and proteins are less available as food for animals, especially ruminants. Scientific researchers, nutritional experts and agronomists are endeavouring to produce more and use that which is available more efficiently to feed animals.

R. Herrera-Saldana and colleagues, working at the University of Arizona (1990), have researched, using lactating cows, the influence of synchronizing protein and starch degradation in the rumen on nutrient utilization and microbial protein synthesis, using barley cereal, milo and cottonseed meal. The latest published research to date (June 1990), is that of McAllister, Buchanan-Smith and colleagues from the Research Station, Alberta and Guelph University, Canada. They have used formaldehyde to regulate the digestion of barley starch in ruminants, and by the use of scanning electron microscopy, have shown how the protein matrix inhibits the access of rumen bacteria to the underlying starch granules. Also, recently in Canada, R.S. Bush has used barley to solve one of the major constraints of early weaning programmes with young calves and has proved unequivocally that barley is, without doubt, the ultimate preference grain in concentrate diets for young calves.

1.2 THE CULTIVATED BARLEY PLANT.

Cultivated barley (*Hordeum Vulgare* L.) is a member of the genus *Hordeum* which belongs to the tribe species named **Triticeae Dumortier**. For over sixty years, the barley plant has been one of the most frequently used among flowering plants for cytogenetic research. Very few plants show such a wide adaptation to the environment as the barley species does. One of the reasons why the *Hordeum Vulgare* species has been successful in its adaptation is because of the versatility and variation of its reproductive system, which has the capacity for cross-fertilization as well as self-fertilization.

1.2.1 Germination.

At the onset of germination, the caryopsis of the barley seed imbibes water and increases in size. As the seed germinates, the coleoptile breaks through the testa and grows up the dorsal side of the barley seed. This occurs at the same time as the radicle and the seedling primary roots commence growing. The coleoptile is a protective sheath, enclosing the apical meristem, with leaf primordia in the embryo and it is specially adapted to force its way up through the soil. Growth ceases when it reaches the surface of the soil. The endosperm contains the majority of storage components (sugars and starch) for the development of the seedling and the embryo controls the release of the endosperm storage components during the germination process. The first true leaf of the barley seedling then emerges at the top of the coleoptile and the barley seedling plant begins to grow and develop (Figure 1.1).

1.2.2 Structure of the Cultivated Barley Plant.

The barley plant consists of:-

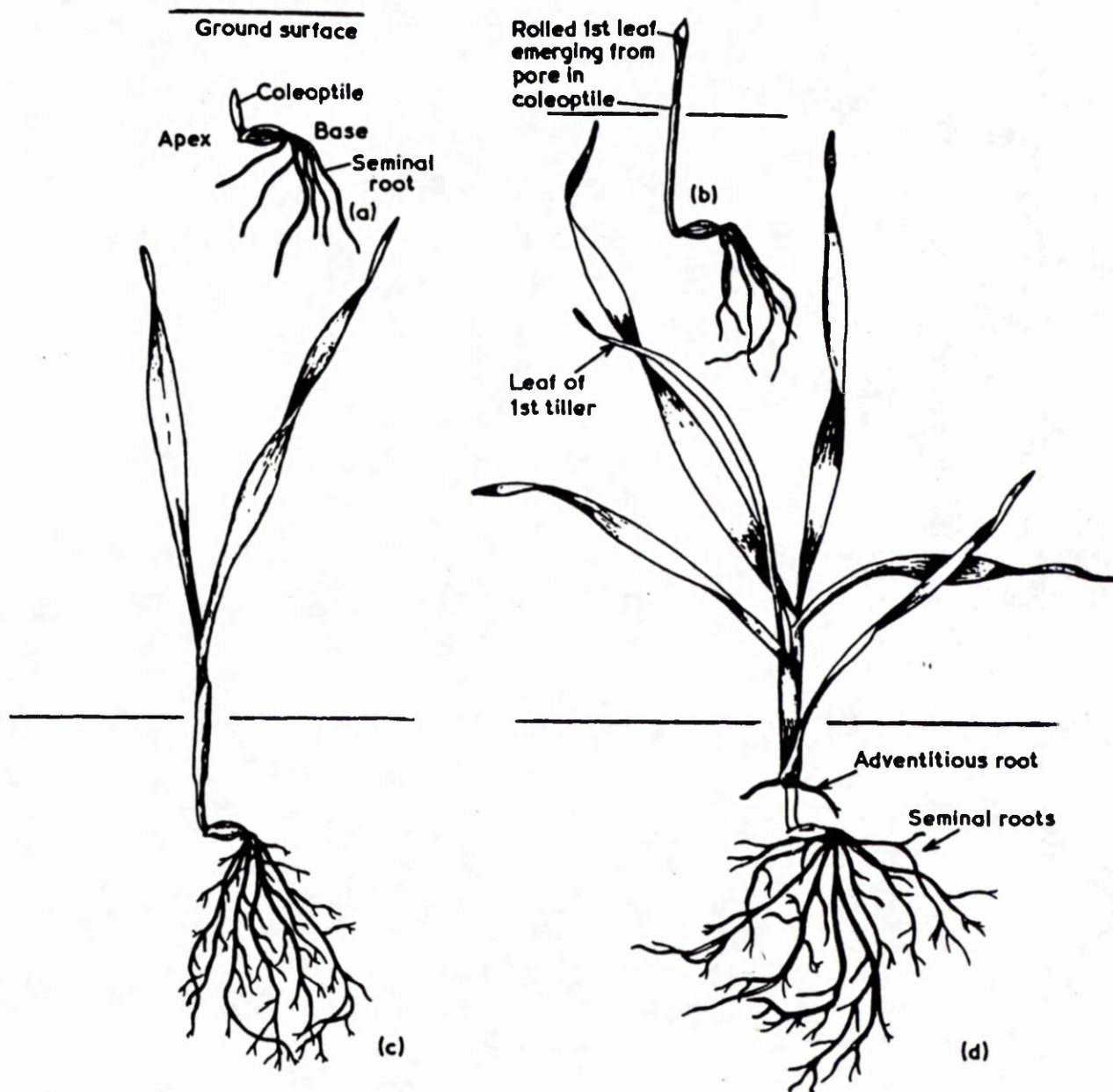


Fig. 1 . 1 Diagrams of the appearance of barley plants at successive stages of growth (after Pedersen [317]). (a) Germinating grain, with the coleoptile emerging at the apex of the grain, the seminal roots at the base. (b) The first leaf emerging from the coleoptile, which has cleared the soil surface. (c) The emergence of the second leaf. (d) The appearance of the first tiller-leaf. The basal region is swelling to form the crown and the first adventitious roots are appearing. (e) The onset of stem elongation (jointing, shooting); one main shoot and three tillers are present. (f) Ears emerged and erect. Two sterile tillers are present. (g) Plants approaching maturity. The leaves are withering and the ears are beginning to nod.

Taken from BRIGGS (1978).

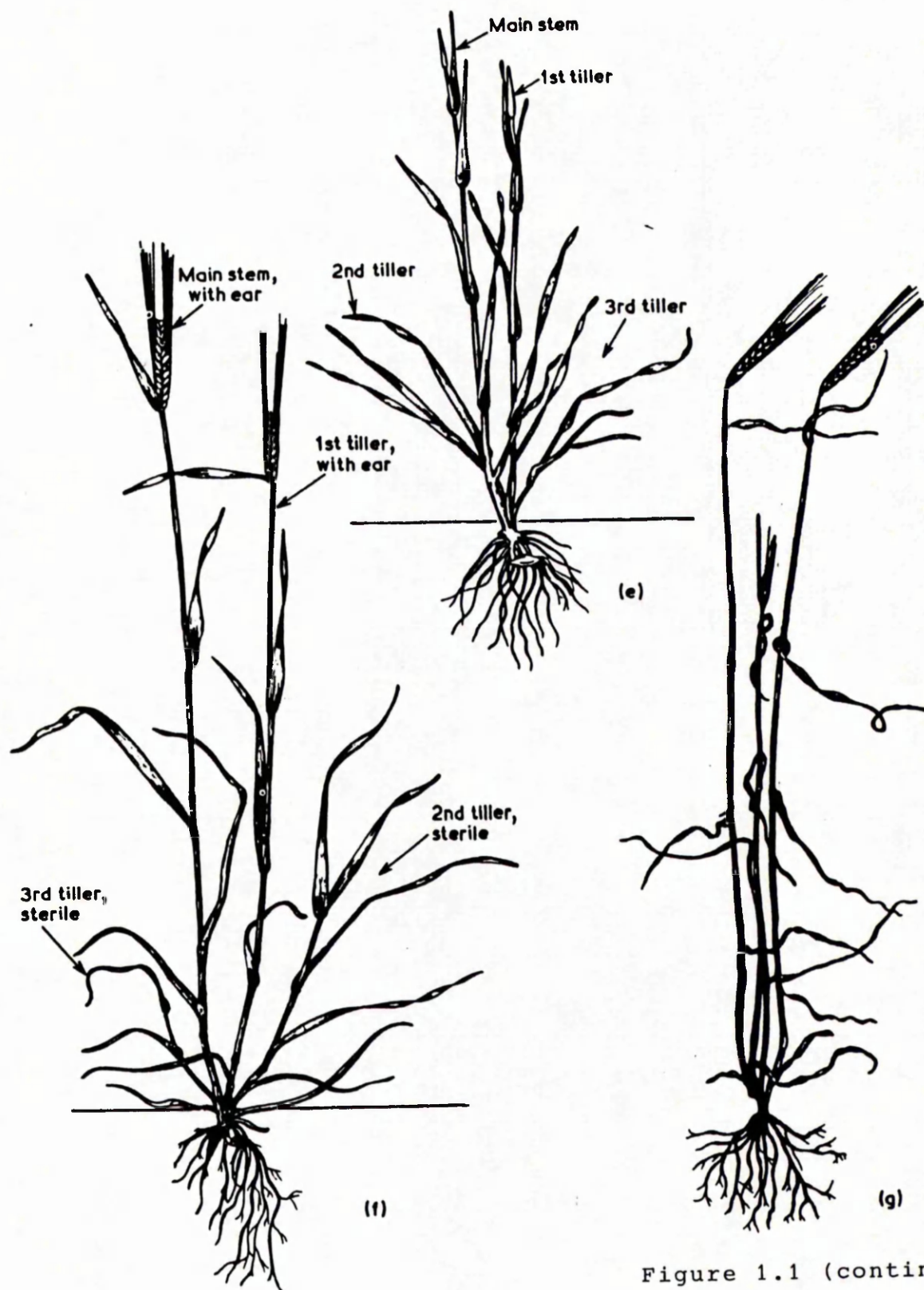


Figure 1.1 (continued).

1. The root.

There are two pairs, the first are the seminal (primary) and the second are the adventitious set.

2. The stem (culm).

This is cylindrical in shape and contains hollow internodes and from five to seven nodes (joints).

3. The leaves.

These develop alternately on opposite sides of the stem above each node.

4. The spike (head/ear).

It is formed at the top of the stem and consists of flowers which are arranged in single flowered spikelets, each of which bear two glumes and the floret.

5. The floret.

This is composed of the lemma and the palea, which enclose the male and female parts of the flower.

6. The kernel.

This consists of the caryopsis in hull-less barley, but in hulled barley the lemma, palea and rachilla adhered to the caryopsis are also included.

The life cycle of the cultivated barley plant can be defined as having a series of three phases. The first phase is characterized by the leaf, stem, spike and tiller initiation. During the second phase, the stem and spike grow rapidly and some tillers and spikes die prematurely. Then grain kernel growth and maturation complete the third and final phase of the life cycle. The growth habit of most cultivated barley is described as Winter, Spring or Facultative.

The root.

At the lower part of the embryo axis is the radical (embryonic

root). It is a simple axial structure. It is not divided into nodes or internodes and does not contain any leaf-like structures. The barley plant develops two sets of roots. The first seminal (primary) set are produced as the seed begins to germinate. They emerge from coleorhiza and between five and seven usually develop, but in some varieties of barley there may be up to nine. The seminal root consists of the epidermis, the cortex and the stele. The root cap protects the growing root as it penetrates the soil. New root cells continuously form behind the root cap and replace the peripheral cells of the root cap as they are worn off. The seminal roots grow downwards and outwards into the soil. They develop freely to form a fibrous, branched mass. The roots develop root hairs, in order to increase their overall surface area to absorb the maximum amount of water and minerals from the soil. The root hairs, which are relatively short lived, develop from the cells of the epidermis. The new root hairs develop along the elongated root, often from cells near the apex. The seminal roots are the deepest ones. They may grow as deep as 1.8m to 2.1m in the soil (Briggs, 1978). Weaver (1926), observed that the seminal roots of a barley plant at the two-leafed stage, had a maximum depth of 25cm and at maturity, the seminal roots were up to 1.4m deep and finely branched. As the tillers develop, the second set of roots, which are the adventitious modal set develop. They emerge from the compressed crown structure which is at, or just below the surface of the soil (Kirby & Appleyard, 1981). The adventitious roots tend to be thicker and less branched than the seminal ones (Briggs, 1978). The adventitious roots occupy the upper layers of the soil. As the roots age a corky layer develops beneath the epidermis. The type and depth of soil, its moisture and nutritive content and

also the genetic variety of the barley seed grain planted, greatly affect the development and depth of all the roots of the barley plant.

The stem (culm).

The stem of barley is cylindrical in shape. It consists of hollow internodes separated by solid nodes (joints) with transverse septa. Usually there are five to seven internodes and the basal internode is the shortest. The internodes increase in length and are progressively smaller in diameter towards the top of the stem. At each node a leaf sheath has its origin. Above each node the leaf sheath is swollen at the meristematic pulvinis. At general seeding rates a plant usually develops between one to six stems. The actual length of the stem is dependent upon the genetic variety and environmental conditions. The length can range from 7cm in the dwarf varieties, to more than 150cm in others. The strength of the plant is also dependent upon genes and environmental factors. The part of the stem at the top of the peduncle, just below the spike, is termed the neck. It is usually straight or gently curved. The transition of the stem into the spike is marked by a structure called the collar. The grain yield of a variety of barley is affected by the stems in a number of ways. As the stem elongates and immediately after elongation, a high proportion of leaf photoassimilate is accumulated, which might have otherwise contributed to the growth of tillers or the spike. The stem also serves as a source of carbohydrates and nitrogenous compounds. These are mobilized and translocated to the kernel during the grain filling period. Greater strength of the stems prevents lodging in the crop.

The tillers on a barley plant grow directly from the leaf axils on the main stem. Usually they grow from the axis of the

coleoptile and the first three leaves. The development of the tiller buds appears to be regulated by the hormone balance of the barley plant. The growth of tillers is supported by the leaves and root of the main stem. The number of tillers formed depends on the genetic variety of the barley plant. In general, two-rowed barley has a higher number of tillers than six-rowed barley. The emergence of tillers does not assure that they will survive. Late formed tillers frequently die prematurely in field conditions. Tiller survival may be dependent on the tiller leaves reaching the higher light levels of the upper crop canopy (Rasmusson, 1985). The number of tillers on the stem of a plant is also influenced by environmental factors. Kirby and Appleyard (1981) stated that long day lengths suppressed tiller growth.

The leaves.

The first true leaf appears from a pore at the top of the coleoptile during germination. A single leaf develops at each semicircular ridge on the stem of the barley plant. The leaves grow alternately, progressively upwards on opposite sides of the stem. The growth of each leaf from primordium to mature length follows a sigmoid rate. In most varieties of barley, the number of leaves on each side of the stem range between five and ten. Some varieties have fifteen leaves or more and some dwarf varieties may develop up to thirty. The biochemical and gas exchange properties and internal leaf anatomy of barley leaves are consistent with the characteristics of those species possessing the C₃ photosynthetic pathway. Japanese researchers have shown that the optimum air temperature for photosynthesis of Winter barley varies between 10°C and 20°C (Rasmusson, 1985). Rasmusson (1984) demonstrated a marked improvement in the grain yield, when the erect leaf character of one variety of barley was

transferred by breeding to another variety.

The mature spike (head/ear).

The inflorescence of the barley plant is a spike at the top of the stem. The spike consists of spikelets, which are attached at the nodes of a flat, zigzag rachis. Each single flowered spikelet consists of two glumes and the floret. The spikelet primordium initiation begins when about half of the leaves on the stem have emerged. The rachis is solid and has alternate nodes and internodes. The length of the internodes may vary from 2mm or less in the dense headed barley varieties to 5mm or more in the lax headed varieties. Three spikelets are attached at each node of the rachis. The three spikelets alternate along the length of the rachis. In the two-rowed variety of barley, only the central spikelet of the three is fertile. In six-rowed barley types, all three spikelets are fertile. When the maximum number of spikelets has been formed, they all grow at the same rate (Rasmusson, 1985). There is a close relationship between the size of the carpel at meiosis and the final grain weight at maturity. The final overall shape of the spike is determined by the space of the mature kernels on the rachis. When the mature kernels are spaced widely, the spike is termed parallel. When the kernels are close together, the shape is either parallel or pyramidal. Frequently, the two lateral rows of kernels on each side of the spike in lax six-rowed barley varieties overlap, giving the appearance of a spike with only four rows of kernels. The position of the spike at maturity varies according to the genetic type of the barley. It may stand erect, or almost erect, or it may assume a curved position. There are all degrees of intermediate expression between the two extremes (Rasmusson, 1985). The final overall length of the spike is variable and the

length is greatly influenced by environmental factors. Spikelet initiation ceases when the awns are formed on the most advanced spikelets. Long awned genetic varieties of barley possess awns which are twice the length of the spike, or they may extend to one and a half times the length. This is the most common type. Awn length of two-rowed barley differs from the six-rowed type. The structure of a typical awn is of a triangular shape in cross-section, but other shapes do occur. The awn is mostly composed of larch parenchyma cells. The largest cells are in the centre of the awn and then decrease in size towards the epidermal layers. Barley awns retain chlorophyll well into the grain filling period.

The kernel.

The mature barley kernel consists of the caryopsis in hull-less varieties of barley, but in hulled barley the lemma, palea and rachilla adhered to the caryopsis are included. The caryopsis consists of the pericarpate guments, aleurone layers, endosperm (starch mass) and embryo. The pericarp, developed from the ovary wall, provides a protective covering for the entire kernel. The embryo within the caryopsis is appressed to the endosperm by its scutellum (cotyledon). At first the barley kernel grows slowly, then it increases to a constant rate. Kernel dry matter is accumulated to a mass of approximately 4mg (Gallagher et al., 1976). Maximum kernel growth rate ranges from 0.9mg to 2.9mg kernel/ day (Gallagher et al., 1976; Scott et al., 1983). The kernels in the central position in the spike have the highest growth rates. Kernel weight is a relatively stable component of the yield in a barley crop. The stability is attributed to the mobilization of assimilate reserves from the stem and other vegetative tissue and this can compensate for a shortage of

current photoassimilate. Kernel characteristics are among the most useful for distinguishing one genetic variety of barley from another, especially when identification has to be made from threshed grain.

1.3 THE NUTRIENT CONTENT OF CULTIVATED BARLEY.

'Nutritional quality of barley refers to a relative merit in providing available food nutrients that are essential to humans and other animals. This encompasses the total nutrients present and the biological balance and availability of these nutrients. Certain components such as fibre and amino acids may be classified as important in determining the quality of barley for certain animal species being fed for a specific purpose.'

[Rasmusson, 1985]

1.3.1 Carbohydrate (starch).

Starch exists in the endosperm of barley cereal, ensiled in a protein matrix (Armstrong, 1972). Starch is the major chemical constituent of the barley kernel and this amount varies inversely with the percentage of protein. Several qualitative genes affect starch content. The gene **am1** on chromosome 2 can produce from 40% to 48% amylose starch. This single recessive gene displays dosage effects in the 3 x endosperm. Another gene, **stb**, on chromosome 7 controls starch. It has a dosage effect in the 3 x endosperm and clumps the starch (Rasmusson, 1985). Differences ranging between 43% and 62% have been found in starch content of different varieties of barley. It is estimated that 10% to 40% of the carbohydrate in the barley kernel is derived from spike photosynthesis (Rasmusson, 1985).

Chemical properties of starch.

Starch is a polysaccharide. The starch of the barley kernel is composed of two major types of molecules, amylopectin and amylose. A minor component termed 'branch amylose' may also be

present. The starch is predominantly amylopectin (74% to 78%), in which linear chains of α -1,4 glucopyranose are branched through α -1,6 chains every 20 to 25 glucose residues (Briggs, 1978). The resulting structure, although compared to a randomly branched tree, is actually highly organized. Estimates of the molecular weight of amylopectin begin at one million. Amylopectin is the only starch in the waxy genetic varieties of barley. The A-chain of amylopectin tends to be short and weakly helical and it forms a weak reddish-brown clathrate with iodine. The degree of branching of amylopectin varies within the genetic types of barley and influences the starch properties. Some varieties of barley exist which contain all amylopectins in the starch. Another variety of barley contains a 1:1 ratio of amylopectin and amylose.

Amylose (22% to 26%) is a linear polymer, containing straight chains of D-glucopyranose units linked α -1,4 (Briggs, 1978). Waxy varieties of barley contain little or no amylose. Amylose exists as a helix, which forms a blue or purple clathrate with iodine. Iodine staining can also be used to distinguish between normal and waxy starches. 'Branched amylose' is thought to comprise 5% to 10% of some starches. Due to the difficulty in isolation, it remains poorly characterized.

Physical properties of starch.

Starch exists in highly organized granules. The amylopectin and amylose molecules are held together by hydrogen bonding. Each barley variety produces granules of a characteristic size, shape and property. Starch granules are insoluble in cold water. The true density of starch varies from 1.4 to 1.6 g/cm³. Granule size ranges from 200 μ m or more. Starch granules rotate the plane of polarized light and exhibit a characteristic shadow, the 'Maltese

Cross' phenomenon, known as birefringence. A starch granule exhibiting birefringence is considered to be in the 'native' state (Rasmusson, 1985).

Granule structure.

Starch granules are pseudo-crystals, that have crystalline (organized) and amorphous (relatively non-organized) areas. The crystalline or micellular region is primarily composed of amylopectin. It is resistant to water entry and enzyme attack and is responsible for birefringence of the granule (Rooney, 1986). Some adjacent A-chains are thought to form a double helix in this region. The amorphous region (gel phase), is less dense than the crystalline area and is rich in amylose. Water moves freely through the amorphous region and amylase attack on the granule begins in this region. Chemical modification of starch, for example cross-binding, primarily affects the amorphous region (Rooney, 1986). Current theories describe starch granules as composed of radial chains of amylopectin molecules, organized in a semi-crystalline cluster arrangement. Granule growth occurs concentrically by simultaneous extension of the amylopectin chains but the exact role of amylose in granule structure is not known (Rasmusson, 1985).

Gelatinization.

The characteristic 'gelatinizative temperature' for starch is the point at which it shows 50% loss of birefringence. Starch granules undergo gelatinization or lack of native structure, when sufficient energy is applied to break the intermolecular hydrogen bonds in the crystalline areas (Lund, 1984; Zobel, 1984). Amylose is leached from the starch granule during the early stages of gelatinization. This indicates that part of it is in the amorphous region. Waxy barley cereal starch produces gel

with unique texture and clarity (Rooney, 1986). Starch has been described as a particularly crystalline, glossy polymer that exhibits non-equilibrium melting behaviour (Rasmusson, 1985). Dilute alkali or acid promotes gelatinization of starch either with or without heating. Mechanical gelatinization of starch occurs during milling or grinding of barley.

Hydrocolloidal carbohydrates, also referred to as β -glucans, are contained in barley in quite sizeable amounts. The β -glucan component in barley varies from 1.5% to 8%. These compounds are mixed, linear polymers of β -D-glucopyranose in which about 25% to 30% of the glucosidic linkages are in the 1,3 position, the remainder being 1,4 (Fleming & Kawakami, 1977). The β -glucans have been quantified in both the aleurone and inner endosperm areas. In barley they form 75% of the cell wall constituents. In the endosperm cell walls the β -glucans are linked to proteins, (possible glutelins) and form high molecular weight molecules (Forrest & Wainwright, 1977). The endosperm cell walls of barley are unique among cereals. They completely enclose the cell, forming a barrier against the action of the proteolytic and amylolytic enzymes. Approximately 50% of the β -glucans can be extracted with water, without the cell wall structure being destroyed (Forrest & Wainwright, 1977). These soluble compounds have been referred to as gums. Acid-soluble β -glucans within a range from 0.4% to 2.3% have been reported in eighteen barleys of different genetic varieties grown in New Zealand and the total β -glucans ranged from 2% to 6.4% (Rasmusson, 1985). Genetic and environmental factors are thought to influence the relative percentage of β -glucans in barley. An analysis of the carbohydrates in the aleurone cell walls showed 23% arabinose, 44% xylose, 2% mannose, 2% galactose and 29% glucose (Rasmusson,

1985). In addition the cell walls contained 16% protein and 1.2% phenolic acids. The carbohydrate portion of the endosperm cell walls of barley analyzed by Ballance and Manners (1978) consisted of 10% L-arabinose, 13% D-xylose, 74% D-glucose and 2.5% D-mannose. Of this material, mixed linkage β -D-glucans represented 70% to 72%. Similar results with two-rowed barley varieties have been reported by other researchers (Forrest & Wainwright, 1977).

Sugar.

The simple sugars found in the cell wall of barley are glucose, fructose and sucrose. Up to 2% of free sugars occur in barley and up to 8% have been reported in certain mutant varieties (Rasmusson, 1985). There are two types of fructans in barley. One is the levan type where the linkage is β -2,6 and the second is of the inulin type with a β -2,1 linkage. Fructans are considered as a substitute for sucrose.

1.3.2 Protein.

The protein content of barley varies inversely with the starch component (Briggs, 1978). It exhibits the greatest fluctuation of the major nutrients. The composition and amount of protein in the barley kernel are two major factors for influencing the nutritional quality of the protein. A higher grain protein percentage has been associated with two-rowed barley variety rather than the six-rowed genetic type. Hull-less types of barley generally have a higher grain protein percentage than the hulled. Tetraploid forms have 30% to 40% higher grain protein in seed than diploid forms (Rasmusson, 1985). The protein content of the cell walls of six-rowed barley has a range of 3% to 5%.

Lysine.

Lysine comprises 5% to 7% of the albumin-globulin fraction and less than 1% of the prolamines in most barley varieties and it is

intermediate between these two levels in the glutelin. The germplasm of high lysine barley is of superior nutritional quality. In all lysine barleys, the improved protein quality is achieved by the change in the relative composition of the protein in the seed with an increase in lysine. With increased protein, there is also an increase in total amino acids and nitrogen.

1.3.3 Lipids.

In most commercial barley varieties the lipid content ranges between 2% and 3%. Up to 4% lipid content can occur (Rasmusson, 1985). Triglycerides form the greatest portion of the lipid in barley. They form from 73% to 79% and are primarily composed of palmitic acid and the unsaturated fatty acids oleic, linoleic and linolenic acids (Briggs, 1978). Linoleic acid is the principal fatty acid of the barley kernel. The greatest portion of the lipid in the barley kernel is found in the endosperm, with a lesser percent of the total occurring in the embryo and hull. Certain lipids are able to complex with the amylose of both native and gelatinized starch. Environmental growing conditions have a very significant effect on the lipid levels in a barley crop.

Ash content.

The ash content of typical barley ranges from 2% to 3%. It is the lowest in the hull-less varieties of barley. The principal mineral contents of barley ash are potassium and phosphorous, with lesser amounts of chlorine, magnesium, sulphur, sodium and calcium. Iron, zinc, copper, manganese and selenium appear in small concentrations in the ash of the barley kernel. Hulled barley varieties contain more calcium and silica than do the hull-less varieties. The concentration of most minerals is often influenced by the soil zone, type and season (Rasmusson, 1985).

Crude fibre.

Cellulose, hemicellulose and lignin are the principal constituents of crude fibre and all are present in the barley plant (Briggs, 1978). The crude fibre content of hulled barley varieties has a range of 4% to 8% with an average of 6% dry matter. Hull-less types of barley average 2% or less.

1.4 PHYSIOLOGY OF THE RUMINANT ANIMAL.

The Ruminantia group of herbivorous animals are those that regurgitate and remasticate their food. They have spacious compartments in the gastro-intestinal tract, where the passage of bulky, fibrous plant food can be delayed, to allow soaking, mixing and fermentation (Swenson, 1984). The herbivorous species of animals have developed intestinal modifications, which enable them to utilize large amounts of cellulose and other plant polysaccharides, such as hemicellulose and xylan. The principal function of the gastro-intestinal tract of the ruminant is to provide for the digestion and absorption of nutrients and the excretion of certain waste products.

1.4.1 Development of the Ruminant Stomach.

The stomach is the most differentiated and most characteristic organ of the ruminant. It comprises of four communicating compartments which are linked from the oesophagus towards the intestine. They are the Rumen, the Reticulum, the Omasum and the Abomasum. At birth, the abomasum is the largest compartment of the stomach of the ruminant and the type of diet in ruminant neonates is similar to that in omnivorous and carnivorous adults. As the newborn ruminant matures, it gradually increases the intake of roughage and the rumen, reticulum and omasum grow rapidly. They reach adult proportions between the ages of six

months and one year.

1.4.2 The Mature Ruminant.

The internal capacity of the mature ruminant's stomach may be as much as 300l (in bovines) and it occupies almost three quarters of the abdominal cavity.

1. The Rumen.

It is the first and largest compartment of the stomach and constitutes 90% of its total mass. It is situated in and completely occupies the left half of the abdominal cavity (Figure 1.2). Anteriorly and to the left of the level of the cardia is the ending of the oesophagus, which continues from the mouth into the rumen and reticulum into the oesophageal groove. The cardia opening is usually submerged in the rumen fluid and ingesta. It remains closed, except when deglutition, regurgitation or eructation occur. A second aperture situated above the cardia (over the rumino-reticular fold), connects the rumen with the reticulum. Liquids and food enter the rumen through the relatively small cardiac opening. The rumen contains 40% of the ingesta present in the digestive tract of cattle and 55% to 85% of that in sheep. The rumen is a multi-compartmented structure and lined with epithelium membrane. The stratified epithelium of the rumen is of the same type as skin, but it is capable of much greater absorption. It is well vascularized and the capillaries are in close contact with the epithelial cells. The absorbent area is greatly increased by the presence of papillae on the epithelium. Symbiotic micro-organisms and billions of bacteria exist within the rumen. The micro-organisms are classified as protozoa, iodophile microbes, micro-iodophiles and ariodophiles (Swenson, 1984). The extensive absorption of nutrients occurs in the rumen.

The Rumen

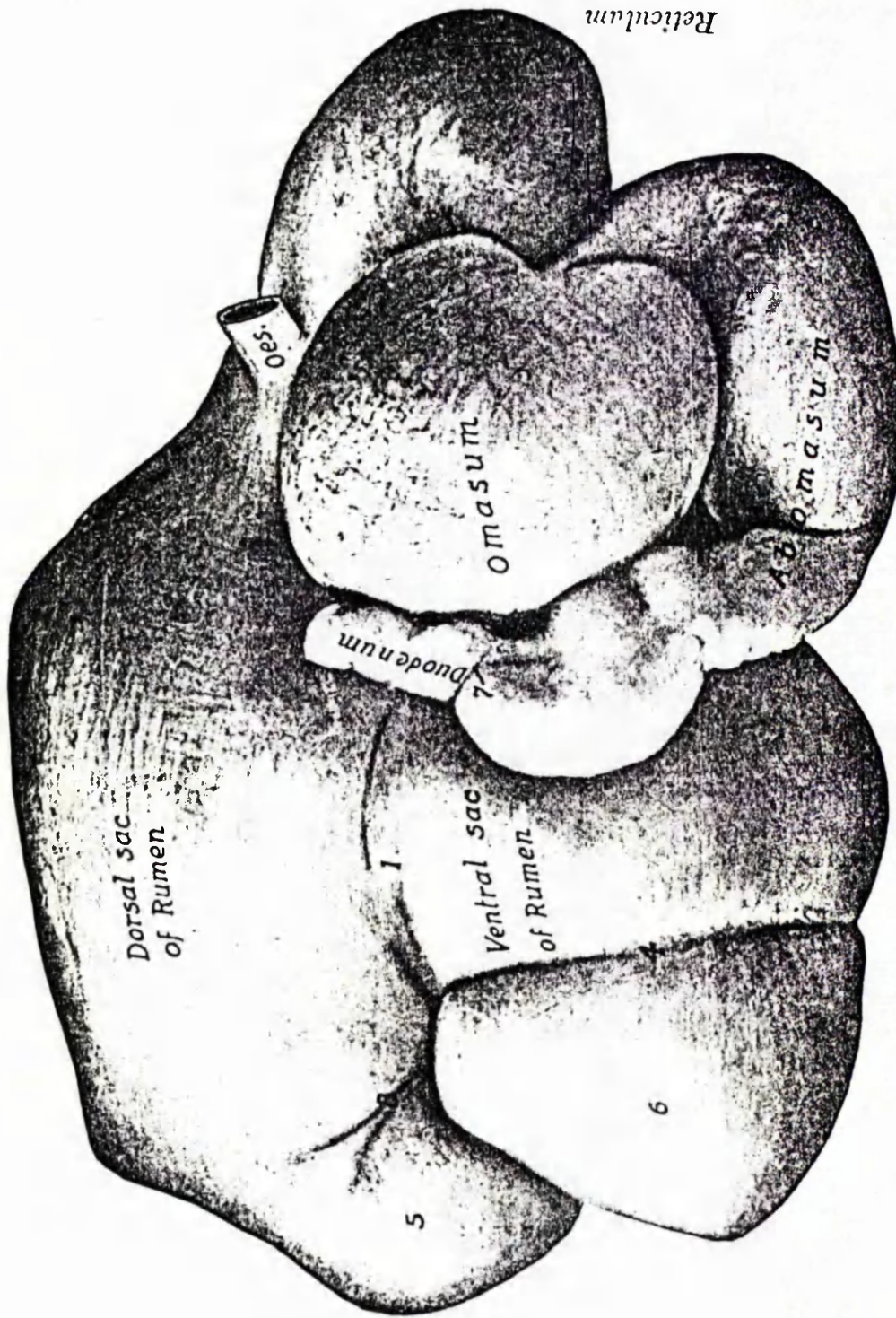


Figure 1.2 Stomach of cow; right view. Oes, Esophagus; 1, right longitudinal groove of rumen; 2, caudal groove of rumen; 3, 4, coronary grooves; 5, 6, caudal blind sacs of rumen; 7, pylorus. (Sisson and Grossman, *The Anatomy of the Domestic Animals*, courtesy of W. B. Saunders Co.)

2. The Reticulum.

This is the smallest compartment of the stomach of a ruminant and it lies in the sub-sternal region, across the median plane. The inner surface is lined with an epithelium membrane similar to that of the rumen. The membrane is divided into a large number of polyhedric alveoli, which resemble the design of a honeycomb. As in the rumen, nutrients are absorbed extensively here and the presence of the alveoli increase the area of absorption considerably. All unabsorbed residues from the rumen and the reticulum are pushed into the omasum through the reticulo-omasal opening.

The Oesophageal Groove.

This is approximately 15cm long and extends from the cardia to the reticulo-omasal orafice and it is a continuation of the oesophagus into the stomach. In young, nursing ruminants this groove becomes a passageway for milk from the oesophagus to the omasum and the abomasum. Owing to the function of the oesophageal groove, the young suckling ruminant behaves almost as if it were monogastric (Swenson, 1984). The micro-organisms responsible for nutrient synthesis have not yet established themselves in the rumen. During the first few weeks of life therefore, the young ruminant depends on an exogenous supply of nutritive proteins and vitamins of the B complex.

3. The Omasum.

It is a more capacious compartment than the reticulum and is positioned deep down in the right hypochondrium. Internally, the omasum is composed of a large number of epithelial lamellae, which occupy the greater part of the compartment. Between these lie deep, narrow furrows, which aid in the transfer of ingesta from the reticulum to the abomasum. Omasal transfer of ingesta

is regulated by the volume of fluid in the rumen, reticulum and abomasum. Nutrients and much of the liquid from the rumen contents are absorbed in the omasum and the particles are reduced in size.

4. The Abomasum.

This is often called the true stomach. It is the only true gastric reservoir to be found in the ruminant animal. It is shaped in the form of an elongated sac and lined with a mucous membrane containing many gastric glands. The abomasum usually holds 7% to 8% of the total stomach contents and is usually kept well-filled at all times. The abomasal motility does not have a rhythmic pattern similar to that of the first three compartments of the ruminant stomach.

5. The Intestine.

The intestine is of great length and lies above the stomach in the right half of the abdominal cavity. The small intestine is coiled round and round. It is approximately 40m in length in oxen and 24m in length in sheep. The large intestine is usually considered as comprising of the caecum, the proximal colon, the centripetal and centrifugal coils and terminates in the rectum. In cattle the caecum is approximately 75cm in length and the colon is 8m in length. In sheep the caecum forms a blind sac between 25cm and 35cm in length and 5cm to 7cm in diameter. The caecum and first region of the proximal colon form a single compartment in sheep, which may contain in excess of 1000g of digesta (Ulyatt & MacRae, 1974). The epithelium of the large intestine of sheep is without villi, but longitudinal folding occurs in the spiral colon and in the rectum. The predominant microflora in the large intestine of sheep (fed conventional diets) are Gram-negative rods belonging to the genera

Bacteroides, *Butyrivibrio* and *Fusobacterium* and anaerobic bacteria are present throughout the intestines (Hungate, 1966).

1.4.3 The Cycle of Motility.

The major cyclic contractions of the rumen, reticulum and omasum, which begin early in the life of the ruminant, continue without interruption throughout its life. The rhythmic, spontaneous contractions only cease during an abnormal situation. The smooth muscle of the rumen is supplied by autonomic motor nerve fibres. The parasympathetic (vagal) fibres are the most important and provide the required innervation for the rhythm of motility in the first three compartments of the stomach. The stomach of the ruminant is also supplied with an extensive network of afferent nerve fibres, which carry information from the receptors in the stomach to the central nervous system. Tension receptors are situated in series with contractile elements in the muscle layer. They are especially numerous in the reticulum and the cranial sac of the rumen. Epithelial receptors are found in the reticulum, the cranial sac and the papillae of the rumen. Epithelial receptors in the rumen and reticulum and the mucosal receptors in the abomasum and duodenum are sensitive to both mechanical and chemical stimuli (Grovum, 1985).

1.5 FEED INTAKE.

'Animal production is dependent on adequate levels of voluntary intake, especially in the case of ruminants which can utilize poor forages and by-products which are of no direct nutritional value to man.'

[Forbes, 1986]

The factors influencing feed intake can be categorized as animal factors and feed factors, although both factors are sometimes inseparable in their action. The animal factors comprise of the genetic breed, the age, size and weight of the ruminant and the

level of milk yield. Included in the feed factors are: the essential nutrient content of the diet, the energy content, the processing of the diet and its ingredients and the special characteristics of the intake (Owen, 1983).

Ruminants have very specialized digestive systems and the features of their digestion are important in understanding the basic principles of feeding ruminants and their young. The transformation of the digestive tract of ruminants from an essentially non-ruminant state at birth to a ruminal state in the mature animal is greatly influenced by the diet it receives.

The ingredients for animal feed can be broadly classified into four main groups:

1. Green Fodders (grasses, clovers, cereals, grazing barley, oats or rye, herbage and weeds).
2. Coarse Fodders, which provide roughage (hay, straw and chaff).
3. Succulents (mangolds, swedes, turnips, potatoes, cabbage, kale and sugar beet tops).
4. Concentrate Foods, rich in carbohydrate and protein (high in proportion of digestible nutrient per unit weight).

Water must also be included as a feeding component. Water, because of its abundance and universal use, is seldom regarded as a feed, but it is one of the most essential nutrients of life.

Maintenance rations.

There is an amount of food the mature ruminant requires to maintain good body conditions and health. Maintenance ration requirements vary according to breed, weight, age, condition of the animal and management. If a ruminant is fed in excess of its maintenance and production requirements, the surplus energy is usually converted into fat. If the animal is underfed, its performance will drop. Satiety for the ruminant after a meal will

become apparent before the real digestion of the food has started.

`Ruminants eat discrete meals, as do most animals.'
[De Jong, 1985]

A normal ruminant will stop eating within a narrow range with regard to its calorific needs.

`Physical control of voluntary food intake achieves an approximate match between intake and energy expenditure, resulting in a fairly consistent adult body weight. Ruminant animals are capable of such regulation provided that the calorific density of the diet is not too low.'
[Baile & Forbes, 1974]

It is believed that many integrated factors bring about the regulation of food intake. Many factors concerning this in man and animals are known, but more research is needed to understand all the aspects of sensations of hunger and appetite.

`It is necessary to stress that nervous regulation is also of extensive importance for the development and inter-relational process of nutrition in animals.'
[Dougherty, 1965]

The response of a ruminant to a given amount of food can vary, depending on whether the food is being used for maintenance, lipogenesis, lactation, gestation or growth. A ruminant should be fed to capacity under all feeding conditions. This process enables the animal to consume enough food to satisfy its hunger and prevents the uneasiness and wasteful excessive movements associated with underfeeding.

`A deficiency of energy due to a simple lack of sufficient total feed is undoubtedly the most common deficiency in beef cattle rations. The results of lower energy intake are slow growth and even loss of weight, stunting and failure to conceive.'
[Snapp, 1962]

A ruminant's appetite governs the amount of food it is able to eat in one day. Animals should not be fed more grain than they can consume in twenty minutes. Both condition and age affect feeding capacity and there is a considerable variation between

animals of the same condition and age. Research has shown that feeding more than 900 g/day of concentrates results in acidosis and lack of appetite in adult sheep. Recent experimental evidence would indicate that the acceptance level of voluntary consumption of a ration is related to the digestible nutrient energy content of the ration, that is, the more digestible the feed, the greater the daily consumption and vice versa. This hypothesis would link feed intake to the quality of the feed, as well as to the size, age or condition of the ruminant receiving the food. Diet is thought to affect rumino-reticular motility through palatability, which affects the frequency of contractions (Grovm, 1985). For a given sheep and food, there was a positive relationship between the amount eaten and persistence of changes in the rumen contractions relative to those observed in the fasted state. The changes were most complete and most persistent for coarse or long roughages, as opposed to those which were ground or pelleted. This implies that the contractions were weaker (Grovm, 1985).

Acetate plays a dominant role in the energy metabolism of ruminants, because of all the volatile fatty acids, it is produced and absorbed in greatest quantities and it may also play a central role in the control of meal size (Baile & Forbes, 1974). Intra-ruminal infusions of acetate solutions of various concentrations before and during a scheduled meal, depress intake in dairy cattle, sheep and goats (Bhattacharya & Warner, 1968). Anil and Forbes (1980) postulated that the liver is of major importance in the control of feeding behaviour and that nervous pathways are important links between the liver and the brain in the control of feed intake.

There is an appropriate relationship between the live weight of an animal and the dry matter content of the food it requires.

A conventional lowland sheep requires a total dry matter intake of about 1.5kg/ 50kg body weight (Mills, 1989). Cows in lactation vary between 2.5 - 2.7kg/ 100kg live weight. Beef cattle tend to consume less than pure dairy breeds. Nurse cows kept for multiple suckling should be fed in almost the same way as commercial dairy cows. Sometimes, undernourished cows which have recently calved often utilize stored body condition to produce milk. Cows that are about to produce calves should have steadily increased rations containing proper supplements and vitamins. Good feed compounds contain adequate minerals for ruminants. Cattle on finishing rations voluntarily consume each day feed in amounts equal to 2.5% to 3% of total live weight. Older cattle such as cows in good condition and fleshy individuals, such as mature bulls, consume less, even as low as 1.5% of their live weight. Growing yearling or older steers may consume up to 3% of their live weight daily. Efficiency of feed conversion and growth rate go hand in hand. The amount of water required by a ruminant, exclusive of the water contained in the feed, varies with the character of the feed, the amount of dry matter consumed and the air temperature.

The demands of young ruminants are greater than those of adults, due to the necessity for synthesis of the increasing cell material (Hungate, 1966). A growing heifer should be well fed to build up her skeleton, to increase her body size and lay down a reserve. This is equally applicable for young sheep. Poor nutrition in the young female ruminant is often responsible for sterility. It may cause difficulties at calving or lambing, or lead to placenta retention.

The chemical and physical properties of forage influence both the quantity consumed and the nutrients which become

available to metabolism in the grazing ruminant (Beever & Siddons, 1985). It has been well documented that the consumption of roughage elicits an early increase in the forestomach capacity, as well as tissue growth and the development of papillae (Dougherty, 1965). A more thorough understanding of the inter-relationship between rumen microflora and the host animal has led to the concept that, in order to feed ruminants adequately, the nutrient requirements of the microflora should first be met. Protein or nitrogen level in the feed ration has a marked effect on the total digestibility of the dry matter of the ruminant feed and especially on the crude fibre. This level is extremely important because of the differences in crude fibre digestibilities, which can have an influence on the digestibility of the remaining nutrients in the feed. The latter might be enclosed in the fibrous portion of the plant.

Young ruminants and those in lactation have such a relatively high need for protein that the maximum production of microbial protein does not supply all their amino acid needs. These have to be supplemented with good quality protein, a proportion of which bypasses the rumen and is absorbed in the small intestine. The use of protein can be optimized by the use of correct ingredients for a feed concentrate mixture. There is considerable variation in the crude protein content of feeds. Nitrogen deficiency in the diet affects ruminants by reducing the activity of the microflora and the supply of amino acids for intestinal absorption. Certain minerals, particularly phosphorus, sodium, potassium, sulphur and cobalt are essential for maximum microfloral activity. High quality alfalfa and certain fermentation by-products reportedly contain components which stimulate the activity of the rumen micro-organisms. The rate of

synthesis of certain vitamins can be altered by varying the level of nutrients in the ration, for example, cobalt. Incorrectly balanced feeding may initiate hypomagnesaemia and acetonaemia, which prevent rumen degradation.

`The level of feed intake dictates the whole rate of metabolism in the body of the cow.'

[Owen, 1983]

`Cattle and sheep digest food at very much the same rate.'

[Dougherty, 1965]

`The composition and availability of the diet are major factors influencing voluntary intake. While the concentrations of protein, the balance of amino acids and deficiency or excess of minerals or vitamins can all affect intake, the major parameter of a food which determines the amount eaten is the concentration of available energy.'

[Forbes, 1986]

`Individual animal differences is probably the most important factor influencing the variation in food intake and rate of gain.'

[Phillipson, 1970]

The importance of feed intake and the factors influencing it are being increasingly realized. However, much further research is required to enhance the ability of all to manipulate different ruminant feeding systems.

1.5.1 The Process of Digestion in the Ruminant.

`Ruminants have very complicated, specialized digestive systems, adapted particularly to utilize a range of forages of varying quality.'

[Owen, 1983]

`The digestive physiology of the ruminant is an integrated system, with each part interacting with the others, from the metabolic activity of the individual species to the mechanical, absorptive and secretory properties of the different parts of the gut wall, and the hormonal control of the metabolism of the products of digestion.'

[McDonald & Warner, 1975]

The main elements of digestion of food in the ruminant are the Mouth containing the teeth, tongue and lips; the Rumen with its microflora; the Abomasum and the parts of the Intestinal Tract.

The duration of the digestion process in the Ruminantia group of animals continues for a long period. The food material enters the mouth. It is manipulated by the tongue, lips and incisor teeth of the lower jaw, biting against the hard, toothless pad of the upper jaw. The food is then masticated by the two sets of grinding molars. As mastication progresses, saliva from the salivary glands situated in the mouth is secreted into the food. This mass of food is deglutinated down the oesophagus into the rumen.

1. Secretion of saliva.

Saliva secretion into the mouth and its subsequent deglutination is of great importance in digestion in the stomach of the ruminant. Ruminant saliva mostly contains a bicarbonate phosphate buffer secreted at a pH of 8.1. Substantial amounts of nitrogen are also secreted in the saliva. Cattle produce between 90l and 190l of saliva daily. A continuous flow of saliva is secreted by the ruminant, which increases in amount with feeding and rumination. Considerably more water enters the rumen through salivation than through water consumed from the food, or by drinking. Also, saliva possesses important anti-foaming properties, which decreases the foaming tendency of certain diets, reducing the occurrence of dietary bloat.

2. The rumen.

A mixture of fluid and food material is contained within the large rumen. The rumen contents are the substrate for a large population of micro-organisms, both bacterial and protozoan. Several types of microbes make up the microbial population of the rumen. The constituents of this population of microflora depend on many factors, the chief one being the diet of the ruminant. The process of anaerobic fermentation which occurs in the rumen

is part of rumen degradation and allows protein and other nitrogen compounds to be degraded to ammonia. Digestion of the feed by this process provides maximum benefit for both micro-organisms and the ruminant itself. The concentration of ammonia is influenced by the quality and solubility of the dietary protein, the quantity of urea that enters the rumen in saliva, the diffusion of urea through the rumen epithelium and the rate at which the ammonia is absorbed from the rumen. Under optimal conditions, much of the ammonia is utilized by the microbes to synthesize their own body protein. Some of the excess ammonia may also be absorbed and then excreted as urea.

3. Volatile fatty acid production.

The products formed during the fermentation process result from the interaction between the microflora and the substrate. The fermentation results in a degradation of the food material, (including cellulose) into volatile fatty acids (VFA), primarily acetic (70%), propionic (20%) and butyric (10%) (Bassett, 1975). The concentrations of volatile fatty acids in the rumen vary over a wide range. Of the organic matter digested in the rumen approximately 80% is fermented to volatile fatty acids. The VFA are absorbed through the rumen epithelium into the bloodstream. Within the body tissue they are metabolized to form the main source of energy for the ruminant. The pH of the rumen contents can considerably modify the interaction between the substrate and the microflora. The proportion of volatile fatty acids can sometimes be related more readily to rumen pH than the composition of the feed. For high concentrate rations, the amount of glucogenic volatile fatty acids would vary widely according to whether high propionate, or high butyrate fermentations developed. High concentrations of volatile fatty

acids occur when ruminants graze new, Summer grass, or when they are fed rich starch diets.

4. Protein synthesis and degradation.

'The first step in protein utilization is digestion irrespective of the use of the protein.'

[Hungate, 1966]

Proteolytic activity occurs on the rumen contents. The bacteria synthesize protein in the rumen by the phenomena of symbiosis. The microflora of the rumen play a significant role in protein utilization. Since they are not specific in their source of nitrogen, or protein, all sources are well utilized by the ruminant. The digestion of protein in the rumen progresses by a steady rate of hydrolysis. This takes place through peptides of decreasing chain length to free amino acids, which are largely destroyed by fermentation deamination.

'When protein is fermented to provide energy, ammonia is formed. The ammonia in turn may be assimilated again into amino acids, but it cannot all be assimilated unless carbohydrate is available.'

[Hungate, 1966]

Ammonia formation is related to the solubility of the protein. The bacterial protein is later digested in the abomasum and intestines and absorbed by the ruminant in the form of amino acids.

Results of *in vivo* studies reported with sheep agree well with the theoretical estimates of 20g crude protein being synthesized for every 100g polysaccharide fermented (Walker & Nader, 1970). Yields of 23g crude protein/ 100g organic matter fermented were found in hay rations, 16g to 23g for various rations of hay and concentrates (Sutton, 1971) and 14g to 25g for purified rations (Hume, 1970a,b). Although about 17g true protein appears to be a reasonable average for most rations, variations in this value may occur that can be related to

different types. Microbial protein is about 80% digestible and a maximum of about 55g glucose can be synthesized in the body for every 100g protein metabolized (Krebs, 1964). Thus the synthesis of 17g microbial protein in the rumen would yield a maximum of 7.5g glucose in the body. The glucose in the rumen is degraded to propionate and absorbed through the epithelium into the blood stream. The utilization of protein within the ruminant tissues is similar to the metabolism of this food in non-ruminants (Hungate, 1966).

5. Vitamins.

The microflora of the rumen are able to synthesize many of the vitamins required by the ruminant animal. B complex vitamins such as riboflavin, niacin, thiamine, pyridoxine, biotin, folic acid and B₁₂ are synthesized in the rumen, sufficient to meet the requirements of the animal. A variation of the level of certain nutrients in the feed ration alters the synthesizing rate of certain of these vitamins. If cobalt, one of the essential mineral elements, is deficient in the feed ration vitamin B₁₂ synthesis is too low for maximum performance by the ruminant. Vitamin K, one of the fat soluble vitamins, is also synthesized by the microflora in the rumen, but the important fat soluble vitamins A, D and E must be supplemented in the food supply to the ruminant animal. The vitamins which have been synthesized are absorbed from the rumen. Certain minerals especially phosphates, sodium, potassium, sulphates and cobalt are essential for maximum microfloral activity.

6. Gases.

The products of fermentation and microbial action in the rumen are not all utilized to maximum advantage by the ruminant. The result of the microbial fermentation in the rumen is not only the

production of volatile fatty acids (VFA) for energy from the carbohydrate material, but also the formation of the gases methane and carbon dioxide. Methane forms 30% to 40% of the total gas produced in the rumen and there is great variability (20% to 65%) in the carbon dioxide produced. Carbon dioxide is evolved during the fermentation of carbohydrates and deamination of amino acids. Both of these gases are normally eliminated from the rumen via the oesophagus by eructation. These gases represent a loss of energy from the food which has been supplied to the ruminant. A large amount of heat is also produced by the fermentation and microbial action in the rumen. Excess heat above that which is required to maintain body temperature is usually of little value, except in the absence of shelter for the animal in extremely cold weather conditions.

Fresh food material is regularly added to the large mass of fermenting rumen contents. A most unique and important feature of rumen digestion is the process of rumination, which plays a crucial role in the digestion of the food material.

7. The rumination process.

Four processes are involved in rumination:

- | | |
|--------------------|---------------------------|
| 1. Regurgitation. | 2. Merycical mastication. |
| 3. Reinsalivation. | 4. Redeglutination. |

A mechanical stimulation in the reticulum, rumino-reticular fold and the rumen area of the cardia initiates the reflex action of rumination. The time spent on rumination is influenced by the coarseness of the feed ration given to the ruminant. On a diet incorporating good quality hay, a ruminant may spend more than 8 hours in rumination. Immediately following the regurgitation process, a bolus arrives in the mouth for merycical mastication. The liquid is squeezed from the bolus and swallowed. The

remastication and reinsalivation of the bolus material occur for a period of approximately forty to fifty seconds and then with redeglutination the process is completed. During the merycical mastication process, an amount of saliva is swallowed two or three times. The redeglutination process occurs approximately five seconds before the subsequent regurgitation of the bolus belonging to the next cycle.

'Rumination is an excellent indication of the good health of a ruminant, because all the many nervous pathways and neuromuscular functions of the stomach must be functioning well for the complex rumination reflex to occur.'

[Swenson, 1984]

The complex, reflex process of rumination is also greatly influenced by stimuli in the environment outside the ruminant. Through the regular cycle of motility, an amount of rumen fluid, containing the smallest particles flows out of the rumen and reticulum via the omasum into the abomasum. The sequence of the contractions in the motility cycle of cattle is similar in sheep.

8. The omasum.

In the omasum, water, sodium ions and carbonic acid (which correspond roughly to the composition of saliva) are reabsorbed. The recovery of salts and water which normally takes place in the large intestine is preceded in the ruminant animal by a preliminary recovery. This allows a plentiful secretion of saliva to be maintained on a circuit well adapted to the microbial life between the salivary glands, the rumen and the reticulum. In addition, the reabsorption of sodium ions by the omasum facilitates acidification by the gastric juices of the abomasum.

9. The abomasum.

The rumen fluid in the abomasum comprises of the bodies of the microflora, which are a rich protein source and undegraded food

material which has escaped the fermentation process in the rumen. The majority of rumen bacteria are destroyed here. The abomasum is the only part of the stomach which secretes digestive juices, hydrochloric acid and several enzymes, especially pepsin. From the abomasum the digesta passes into the small intestine.

10. The small intestine.

In the small intestine the digestive process continues. Absorption of amino acids and carbohydrates take place through the wall of the small intestine and lipids are degraded. Amylase and maltase are secreted by the small intestine and the pancreas. Lactase and dextranase are also secreted by the small intestine. Digestion is continued as the fermentative digesta flows from the small intestine into the large intestine.

11. The large intestine.

Digestion still continues in the large intestine. The fermentative digestion in the caecum does not differ greatly from that in the rumen. In sheep, in the single compartment formed by the caecum and the proximal colon, which is the main site of fermentative digestion, the passage of digesta is delayed. There is little mixing of digesta once the spiral colon has been reached. The nature of a diet can greatly affect caecal motility in ruminants. Food intake has an effect on digestion retention time in the large intestine, retention time decreases as intake increases. Digesta entering the caecum is composed of refractory residues of the diet, undigested products of rumen fermentation, endogenous secretions and cellular debris from the intestinal wall. Variations in caecal digesta composition do occur due to the intermittent filling and emptying of the compartment, but a relatively constant environment suitable for the maintenance of a complex, microbial population is maintained. The microbial cells

synthesized during the fermentation are not digested by the ruminant. Digestion in the large intestine accounts for between 4.2% and 26% of digestible energy (MacRae & Armstrong, 1968; Beever *et al.*, 1970, 1972; Ulyatt & MacRae, 1974). Volatile fatty acids are absorbed from the caecum of ruminants (Barcroft *et al.*, 1944) by simple diffusion through the epithelium. The molar proportions of the volatile fatty acids produced in the caecum have higher acetate and lower butyrate than found in the rumen. Caecal volatile fatty acids contain a relatively high concentration of branch chain acids compared to the rumen, which is an indication of a greater conversion of protein to volatile fatty acids (Orskov *et al.*, 1970). The caecum accounts for between 8.6% and 16.8% of total volatile fatty acid production in the ruminant animal. When absorption has taken place, the undigested material remaining in the large intestine is excreted via the rectum as faecal output.

1.5.2 The Digestion of Carbohydrate.

Carbohydrates are an essential component of the diet of all animals. The carbohydrate component of ruminant diets can be divided into two groups, the first consisting of the so called available carbohydrate, which is available not only to the ruminant but to all animals. This group can also be considered as the intracellular carbohydrates. The second group consists of the unavailable or partly available carbohydrates. This group ... can be degraded and utilized to a large extent by ruminants. These carbohydrates are the structured polysaccharides of the plant cell wall and are commonly referred to by nutritionists as 'fibre'.

[Morrison, 1979]

The ruminant is able to digest and utilize a wider range of carbohydrates than most other mammals.

[Sutton, 1971]

The first digestive process which carbohydrates undergo takes place in the anaerobic fermentation in the rumen. Carbohydrate is the chief energy supply for anaerobic micro-organisms.

'Of the digestible feeds, carbohydrates are by far the most important quantitatively, because of their superiority as a source of energy under anaerobic conditions.'

[Hungate, 1966]

Polysaccharides are hydrolyzed to dextroses and pentoses. These are then oxidized to acetic acid and various reduced products, in particular propionic acid, butyric acid and methane. The proportions of the products vary widely. It is thought that all carbohydrates are fermented by a common pathway, the Embden-Meyerhof glycolysis system to pyruvate (Sutton, 1971). The fermentation of carbohydrate is arrested by a lack of nitrogen. The relationship between carbohydrate fermentation and nitrogen assimilation varies with the changes in the variety and ratios of feed available to the ruminant. The interaction between the substrate (food), microflora and rumen pH are complex and the proportion of volatile fatty acids formed in the rumen is unpredictable, particularly when high concentrate rations are given. The fermentation of carbohydrates provides energy to support microbial growth which provides the principal source of protein for the ruminant. Wide variations occur among the species of bacteria in the amount of protein synthesized per unit of carbohydrate fermented (Hogan & Weston, 1970).

'Microbial protein synthesis is likely to be related to the quantity of carbohydrate fermented.'

[Hungate, 1966]

When forages are given alone (in the chopped or long form) more than 85% of the digestible fibre and 90% of the total soluble carbohydrate are digested in the rumen. 40% to 50% of the carbohydrate proportion of the food supplied to beef cattle may be converted to volatile fatty acids and absorbed directly from the rumen, after which they are metabolized for energy or stored as fat. Increasing quantities of starches and sugars in the diet

decrease the concentrations of ammonia in the rumen. Carbohydrates escaping fermentation in the rumen may produce from just under 0.5 to 0.75 of the possible glucose sources. Part of this would be structured carbohydrate which would be fermented in the lower intestine. Insufficient carbohydrate may result in the disease metabolic ketosis.

Fibre.

It is desirable to maximize fibre digestion in the rumen, as both the voluntary intake and overall digestibility of forages are limited by the rate and extent of ruminal fibre digestion. However, in modern production systems supplementation of forage-based diets with cereals reduces fibre digestibility (particularly cellulose) and this can lead to a reduction in forage intake (Orskov et al., 1978). A change from fibrous to concentrate feeding must be made very gradually, often over a period of 2 to 3 weeks, to allow adaptation by the rumen microbes (Orskov, 1987). Cone et al. (1989), showed that the degradation of starch from the same feed in rumen fluid from a hay-fed cow, was significantly lower than in rumen fluid from a concentrate-fed cow. Addition of readily fermentable carbohydrate to a high fibre diet changes a range of rumen parameters. Hoover (1986) proposed three mechanisms to account for this.

a) The carbohydrate effect.

As starch and sugars are rapidly and easily digested in the rumen, it was proposed that these are preferentially degraded by rumen micro-organisms and act as a competitive inhibitor of fibre digestion. The fermentation of cell constituents (hemicellulose and cellulose) often does not commence until around six hours after feeding. It was proposed that the fermentation depended on colonization of the plant cell walls by bacteria, protozoa and

fungi (McDonald et al., 1988; Van Soest, 1982). The preference for a readily fermentable energy source by the rumen microbes is supported by the work of Mould and Orskov (1983). They observed that cellulolysis was only partly restored in sheep fed a concentrate diet when the pH was increased by infusion of a bicarbonate solution.

b) *The pH effect.*

A decrease in rumen pH, both *in vivo* and *in vitro*, depresses fibre digestion. Mould and Orskov (1983) showed that as pH was gradually reduced from 6.8 to 6.0, only small depressions of fibre digestion were observed. A further decrease to below 6.0 caused severe inhibition in cellulose digestion. The pH of 6.1 - 6.0 has been therefore designated as the 'cellulolysis threshold', below which the depression of cellulolysis is significant (Mould & Orskov, 1983). This depression is related to both the extent of pH reduction below 6.0 and to the duration of the depression. Severe reductions in rumen pH (< 5.5) are normally linked to the accumulation of lactic acid, which can cause a serious reduction in fibre digestion rate.

c) *Competition for essential nutrients.*

Under conditions of high fermentable carbohydrate (and also limiting protein), the rapid growth of amylolytic and saccharolytic organisms may reduce the availability of nutrients such as ammonia, amino acids and peptides to the cellulolytic organisms. Fibrolytic microbes inhabit specific micro-environments within the rumen and can therefore experience deficiencies, even when the total nutrient concentration in the rumen appears adequate.

As the proportion of forage to concentrate in a ration decreases, the molar proportion of propionic acid tends to

increase. This is accompanied by a fall in the molar proportion of acetic acid and is described as the establishment of a propionate fermentation pattern (Sutton, 1971; Mould et al., 1984). The reduction in acetic acid, due to the inhibited activity of the cellulolytic microbes, can lead to a decrease in milk fat. This has serious implications for the producer, as efforts to increase lactation by feeding more concentrates can be hampered due to the lower milk fat content, giving reduced energy output in the milk (Orskov, 1987).

Starch.

Within feed rations consisting mainly or even entirely of concentrates, starch is often the most abundant carbohydrate. The structure and composition of cereal starches and their interaction with protein plays a major role in the digestibility and feeding value of grain for ruminant animals. Starch is degraded by the action of a variety of amylases. These enzymes are present in the saliva of many animals and are secreted by the pancreas. Starch degrading enzymes are also produced by a large number of different rumen microbes especially *Bacteriodes amylophilus*, *Succinivibrio dextrinosolvens*, *Selenomonas ruminantium* and *Streptococcus bovis*. As well as the bacteria, rumen protozoa, particularly oligotrichs, will ingest food particles and they can even use complex carbohydrates (Morrison, 1979). The mechanics of starch fermentation by rumen bacteria has been studied extensively. Certain principles regarding sites of digestion were established some years ago by Karr et al. (1966), Orskov et al. (1969) and Beever et al. (1970). These were that when barley, wheat or oats are fed to ruminant animals either as whole, or processed grain, at least 90% of the starch is fermented in the rumen. French (1973), Waldo (1973) and

Galyean *et al.* (1976), also found that 90% to 95% of the starch of most cereals is digested in the rumen by the rumen bacteria and most of the remainder in the small intestine. Starch is digested rapidly in the rumen, but more slowly than the sugars (Hungate, 1966). Waxy barley starch is digested as equally well as, but no better than, starch from normal cultivated barley (Calvert *et al.*, 1976). It has been noted that even though a rumen pH of 6.8 was optimal for *in vivo* starch utilization, starch fermentation could occur at a rumen pH far below 6.0 (Ravelo *et al.*, 1988). Unlike cellulolytic bacteria, starch fermenters are quite insensitive to acidity. They ferment starch equally well at pH 5.5 as at pH 7 (Orskov, 1987). Raw maize is an exception, 25% or more may escape digestion in the rumen. When large amounts of maize are given some may even reach the caecum. Cereal starches are the most easily digested. A wide variation in the digestibility of isolated starch granules *in vitro* and *in vivo* has been reported by researchers (Dreher *et al.*, 1984). The digestibility of starch is generally inversely proportional to the amylose content.

'Starch in common with soluble carbohydrates, exhibits a high percentage of conversion into fermentation products and cells. The undigestible residues are minor.'

[Hungate, 1966]

In a review of individual grain studies, Waldo (1973) and Spicer *et al.* (1986) indicated that barley cereal starch and protein were digested more easily by cattle than those of sorghum. Kay *et al.* (1972), also reported that barley cereal starch was more digestible in the rumen than maize starch.

α - Amylase.

Salivary α -amylase and pancreatic amylases are of critical importance in the digestion of starch (Rooney, 1986). The linear

and branched dextrans produced by α -amylases and debranching enzymes are hydrolyzed much faster by intestinal amyloglucosidases than are large starch molecules. Therefore, the yield of glucose from the digestion of starch feeds is increased by their action (Rooney, 1986).

'The quantities of starch absorbed from the small intestine may also have important implications as far as enzyme adaptation is concerned and the results obtained may suggest that the ruminant is capable of becoming adapted to secrete large amounts of amylase than has hitherto been realized.'

[Orskov et al., 1971]

Faichney (1977a) found in his research using sheep, that of the starch entering the small intestine 82% to 89% was digested there and 11% to 18% disappeared from the large intestine. The carbohydrate reaching the caecum is less digestible than that entering the rumen. The α -linked glucose polymers passing the abomasum are not always completely digested in the small intestine, but substantial quantities may disappear through fermentation in the large intestine, thus affecting the type of fermentation and microbial nitrogen synthesis at that site (Orskov et al., 1970). From their research with sheep Orskov et al. (1970) stated, that if large quantities of digestible dietary carbohydrate escaped ruminal fermentation and reached the caecum it is not of advantage to the host animal because if large quantities are fermented there, the absorption of volatile fatty acids is inefficient and the microbial nitrogen formed is not digested.

Glucogenic material.

As a result of microbial fermentation in the rumen, little glucose is derived directly from the intestinal tract of the ruminant animal, except possibly where diets containing large amounts of maize are fed. The glucose metabolized by their

tissues is therefore derived mainly via the gluconeogenic pathways from propionate and amino acids or by the recycling of lactate and glycerol.

'Ruminants utilize almost as much glucose on a body weight basis as do other species when compared in the basal state.'

[Bassett, 1975]

Ruminants are almost totally dependent on the gluconeogenic pathways for the provision of glucose in the fed state as well as during fasting. It is assumed that only 5% of ingested starch enters the duodenum and that all of it is hydrolyzed to glucose. However, with high proportions of maize in the diet, up to 25% of the ingested starch can leave the rumen unfermented and of this, 75% is hydrolysed to glucose in the small intestine. The remainder is fermented to volatile fatty acids in the terminal ileum and caecum. A comparison of three situations when maize was given emphasized that the type of fermentation in the rumen can increase the total supply of glucose precursors to a greater extent than can a large change in the site of digestion of starch (Sutton, 1971). According to estimates of Lindsay (1970), the entry rate of glucose into the tissue would be about 110 g/d at maintenance energy intake. McAllan and Smith (1974), using calves, estimated that about 100g bacterial α -dextran glucose per day entered the duodenum. For an assumed digestibility of 65% this corresponded to an entry into the body of about 65g glucose per day. They also stated that nearly all the rhamnose, mannose and ribose and about half the galactose and glucose came from bacterial synthesis, whereas nearly all the arabinose, xylose and cellulose-glucose was contributed by the diet. It is only when cracked maize is given, that the uptake of glucose from the entire gastro-intestinal tract provides more than about 15% of this total. With other feed rations, propionic acid and protein

would have to be converted to glucose with an efficiency of about 50% to 70% to meet the differences between the amount of glucose absorbed from the gastro-intestinal tract and the estimated entry rate.

The experiments which have been conducted with adult cattle suggest that the relative importance of the different parts of the whole gastro-intestinal tract for carbohydrate digestion is broadly similar to that of adult sheep. Both cattle and sheep are able to digest completely the starch contained in cereal based diets (MacRae & Armstrong, 1968).

1.6 THE UTILIZATION OF CULTIVATED BARLEY IN RUMINANT FEEDING.

Cultivated barley is used principally as a feed source for animals, as a major ingredient for the distilling and brewing industries, as food for human consumption and for seed. In addition to the uses of the grain, the straw is also utilized for fibre and animal bedding. Young barley plants may be grazed in pasture, harvested as a forage crop, or cut for hay and silage. The predominant use of cultivated barley cereal grain is in providing the major source of energy in concentrates for ruminant livestock and in the feeding of poultry and pigs. Nutritionally, the kernel of the barley is the most important as an energy source, because it is rich in starch, although quite low in oil. Barley contains structural carbohydrates which are defined as 'crude fibre'. The total quality of the fibre is determined by the absence or presence of the hull (husk). Barley fibre has little or no energy value for non-ruminant animals and certain of the carbohydrates, which are water soluble, may create digestive problems, especially in poultry.

Barley may be presented in a variety of ways to provide feed for ruminant animals:

1. The grain.

It may be fed as the sole cereal grain, or blended with other cereals, soya bean meal, cottonseed meal etc., to make up concentrate rations. Primarily, barley grain supplies carbohydrate and protein in the feed ration. High protein content is desirable in barley used for feed. The protein content of barley (80-120 g/kg DM), is generally comparable to the protein content of wheat grown in a similar environment and is slightly higher than rice, maize or sorghum. In addition to being an energy source and supplier of protein and fibre, barley cereal also contains variable amounts of nutritionally important vitamins and minerals.

Processing of the feed.

Barley requires processing to achieve optimal feeding results and needs to be supplemented with other feed ingredients to provide a palatable feed, along with the proper balance of essential nutrients. The processing method is a major factor that can increase the availability of plant nutrients to the ruminant animal (Armstrong, 1972). The application of processing may take many forms.

1. *Physical processing.* There are eighteen recognised methods, the most popular are: grinding, rolling, pelleting, steam-flaking, crimping, popping, micronizing and synchronizing.
2. *Chemical processing,* for example, alkali treatment.
3. *Special processes,* for example, ensiling high moisture grains, protection of the protein fraction.

Processing methods obviously affect feed intake through their effect on the protein value in the diet of the ruminant. The

processing of cereal grains has a significant effect on their intake and digestibility.

'It may be that processing methods have a marked effect on the disruption of the protein matrix and permit easier access of bacterial or animal enzymes to the starch granules, rather than the effect been entirely that of disruption to the starch granules due to processing, however the direct effect of processing upon the starch of the grain cannot be discounted.'

[Hale, 1973]

It is generally accepted that barley should be coarse ground, crimped or rolled prior to feeding, if its full potential as a feed for dairy cows and cattle is to be realized; although this is not necessary for sheep (Orskov, 1986). Barley that is too finely ground can result in unpalatability (Armstrong, 1972; Theurer, 1986). Animals fed on ground grain diets spend less time eating and ruminating. These are both important activities which stimulate the production of saliva. The decreased buffering effect of saliva and the high production of VFA, due to high fermentability of the diet, cause a depression in ruminal pH. This can lead to acidosis, parakeratosis, clumping and necrosis of ruminal papillae. Bacterial invasion of the portal system may also occur, resulting in the development of liver abscesses. The lack of fibrous structure in finely ground diets leads to lack of tactile stimuli, inadequate abrasion of the ruminal epithelium and reduced ruminal motility (Orskov, 1986). When barley cereal is mixed with molasses, palatability is enhanced. Variabilities in protein and amino acids are the major differences in the feeding values between waxy and high amylose varieties of barley and normal varieties of barley (Newman et al., 1978).

2. Barley by-products.

The barley by-products of the brewing and distilling industries, notably spent dried grains known as Draff and culms from mature barley, along with surplus yeast, are utilized extensively for

ruminant livestock feed.

3. Barley as a forage crop.

There are differences in the nitrogen content of the leaves at different plant growth stages of barley. The highest nitrogen content occurs at the seedling growth stage. Researchers have discovered that genetic varieties of barley differ for straw nutritional quality. Significant differences occur for *in vitro* dry matter digestibilities, percentage of crude protein, total cell walls, hemicellulose and uronic acids, fibre, ash, silica, calcium and magnesium content in barley varieties (Rasmusson, 1985).

'The extensive use of forages as energy feeds for ruminants is based on microbial digestion of structural carbohydrates, such as cellulose and hemicellulose.'

[Armstrong and Beever, 1969]

4. Barley hay and silage.

Barley is now increasingly grown as a silage crop. It may be ensiled at moisture levels of 25% to 30%. The dry matter content of the crop increases from about 190 g/kg to about 430 g/kg as the plant matures (McDonald, 1981).

The nutritive contents of barley are higher than those found in maize, reaching peak values at the 'mealy ripe' stage of growth. High concentrations of glucose and fructose are present in the initial stage. As the barley plant matures, the relative amounts of these decrease and fructose containing oligosaccharides and fructans increase in concentration, until the kernel stage is reached. For maximum preservation and nutritive value, the barley crop designated for silage should be cut when it has reached the stage between the 'milky' and 'mealy ripe' stages of growth (Appendix A).

It is possible to treat the barley silage crops with additives prior to storage. These additives, classified as fermentation stimulants, are designed to stimulate the development of lactic acid and increase the content of soluble carbohydrates. Formic acid is predominantly used, but sulphuric acid is used also. They are both used in combination with formaldehyde. The beneficial effect of formic acid in improving the voluntary intake of dry matter is well established and documented (Waldo, 1973). The comparisons between treated and untreated silages in relation to animal performance can be very significant. The tower shaped silo is the preferred type of structure for ensiling the barley crop. The main disadvantage of whole crop barley, as with other cereals, is the fact that it is low in protein content.

5. High moisture ensiled barley.

Barley cereal with a high moisture content stored by ensilation methods, or processed and stored with a preservative, provides a valuable concentrate food source for all ruminants. Barley with a high moisture content may also be ensiled as part of a mixed crop with peas, beans or oats, to provide ruminant feed. In this type of feed the ruminant receives soft, moist, barley grains which have been treated with an additive, in its concentrate rations. Kennelly et al. (1988a,b) concluded that the feeding value of barley with a high moisture content was equivalent to that of dry, mature barley on a dry matter basis for cows in lactation. Feed from high moisture ensiled barley reduces the incidence of digestive problems (Mathison, 1981; FSL Bells, 1989). The relative value of barley in the high moisture state is dependent upon the storage system, the stage of feeding and the source of the roughage in the diet.

1.7 HIGH MOISTURE BARLEY.

Ensiled high moisture barley is now considered as a replacement for dry mature barley in the concentrate feed rations for ruminants. High moisture barley is cultivated barley which has been harvested directly by the combine harvester earlier than the traditional harvesting period. This is when the barley crop has reached 21.5% to 47.8% moisture content, compared to mature barley grain with a 10% to 14% moisture content. Agronomists have found that barley is physiologically mature at 30% to 40% moisture. The starch and protein deposition in the kernel is essentially completed when the moisture content reaches about 40% (Krall, 1969; Rasmusson, 1985). The cultural practices prior to harvesting barley of a high moisture content do not differ from those commonly practised for dry, mature barley grain production.

Harvesting.

The harvesting of barley with a high moisture content should commence when 50% to 60% of the cultivated barley crop has reached the required stage. The barley kernel should be in the 'dough' stage of maturation (Appendix A). At the high moisture stage, dry land cultivated barley stands 3cm taller than mature cultivated barley and it can be cut at least 6cm higher than mature barley (Figure 1.3). In the high moisture state the barley kernels do not disintegrate easily. Mature, dry barley kernels are more likely to crack and break as they pass through the combine harvester. The differences in the size and in the consistency of the kernels of high moisture barley are dependent on the environmental and climatic conditions which prevail during the harvesting period. When barley in the high moisture state is harvested, the health hazard of dust is reduced to a minimum and so also is the danger from fire. Greater yields can be obtained

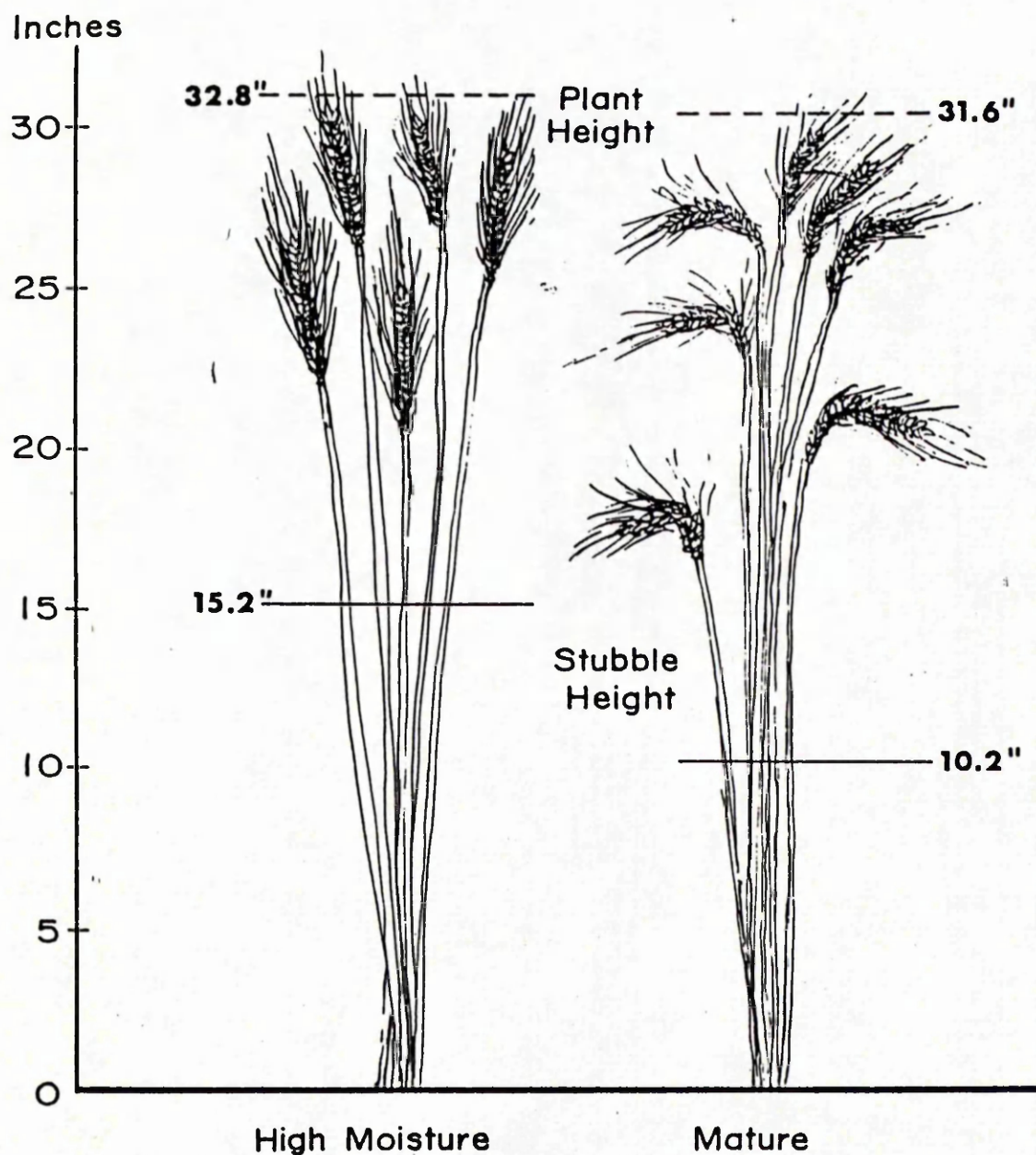


Fig1 . 3 Differences in plant and stubble height of high moisture and mature barley. The nodding head characteristic of mature barley requires the cutter bar to be lower and increase the swath thickness at harvest compared to high moisture barley.

(1 inch = 2.54cm).

Taken from KRALL (1969).

when cultivated barley is harvested at the high moisture stage (Fletcher, 1988; FSL Bells, 1989). An average increase in yield of 6.7% was reported by Krall (1969), in his extensive research trials in Montana, Canada. High moisture barley contains 1.6% more crude fibre than cultivated barley at the mature grain stage. The resulting straw is also better in feed value for animals.

The Ensilation Process.

High moisture barley can be ensiled successfully (Hyde & Oxley, 1960; Krall, 1969; Jones et al., 1974). The storage of cultivated barley grain in the high moisture state is more complex than the storage of dry, mature grain. To guarantee success, the ensiled barley must contain sufficient moisture to ensure anaerobic preservation. A moisture level of between 35% and 45%, preferably over 40%, is required. To avoid spoilage, air and oxygen must be excluded so that the harmful micro-organisms and oxidation will not decompose the barley grain. Research reports on the ensilation of cultivated barley at the high moisture stage are limited, but barley grain in the high moisture stage has been ensiled successfully in silos and bins, open pits, small sealed containers, nylon reinforced butyl rubber bags and airtight silos (Figure 1.4). In the Montana research trials, 45lb per cubic foot (approximately 680 kg/m³) was computed as the storage space requirement for firmly packed barley with 35% moisture content (Krall, 1969). The problems arise when these containers are opened for use, although a specific quantity of the surface material is used daily for feeding. Fewer difficulties are encountered when the high moisture barley is processed and treated with a preservative additive prior to ensilation. Specialized mills have been

Sealing Bunker Silos

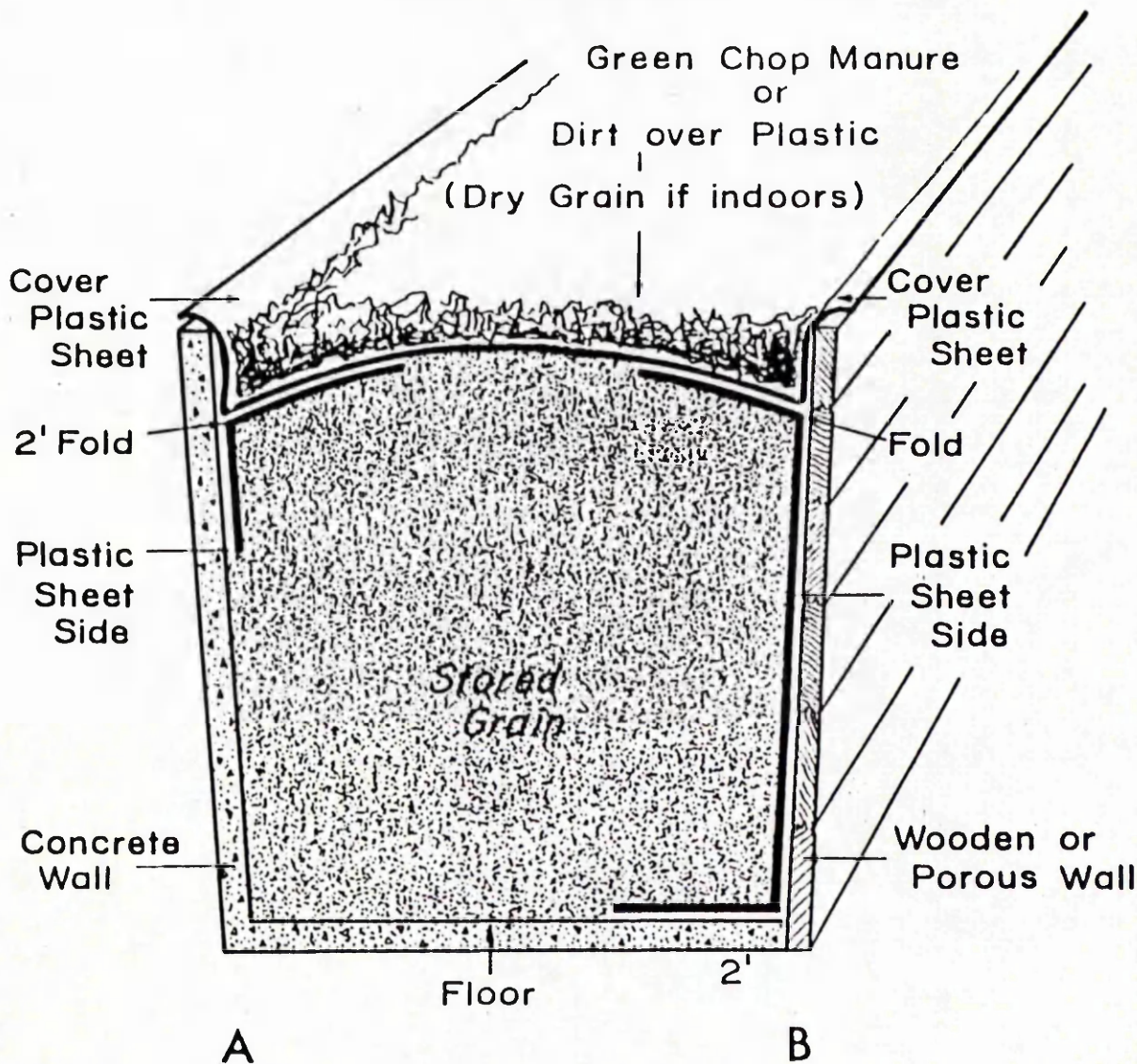


Fig.1.4 Method used in sealing high moisture grain in bunker silos. (A) **Treated Concrete Wall:** Plastic sheeting is placed along side starting two feet from the top of wall then grain is packed against sheet, remainder of sheet folded over grain. Cover sheet is placed over both folds then weighed down with heavy material. (B) **Wooden Walls** are same as A except sheeting extended to floor and two feet under grain. Sheet must be in place before filling.

(1 foot = 30.48cm).

Taken from KRALL (1969).

designed to process cultivated high moisture barley, but modified roller, hammer or burr mills will satisfactorily process the high moisture barley grain. The flattened kernel, plus kernel particles in crimped/ rolled barley cement together more than do whole kernels. This cementation effect is important, because it assists the sealing of the ensiled barley grain. When opened for feeding, crimped/ rolled grain does not deteriorate and spoil as easily as the whole grain barley. Also, for efficient utilization by ruminant animals, grain kernels must be broken in some way to expose the starch and protein to the action of the preservative and the digestive enzymes. The processing of the high moisture grain designated for ensilation should be carried out immediately after the completion of the harvest stage. The high moisture Atem genetic variety of barley used in this research had undergone a complete crimping (bruised/ rolled) process. Crimping is a cold process whereby mechanical force is used to crack open the barley grain, thereby exposing the starch granules to enzyme attack. The grain is not rolled completely flat as in a conventional mill. The problem with rolling is, in order to crush the smallest grains, the large grains are over-processed. It is therefore desirable that the smallest grains are not processed so as to ensure the optimum amount of crushing for the rest. Crimping relies on breaking the grains and is more effective than rolling, since there is no problem with grain size (Orskov, 1987). The crimping process was carried out by a roller mill of special Finnish design. This was the 'Murska' Crimping Machine, with a unique dimpled roller designed especially for high moisture grain.

Commercial additives have been designed and developed to assure the maximum response from a crop, to prevent the problems

associated with fermentation and to allow the grain to be stored under similar conditions as those required for dry, mature grain.

`Graintona Plus' Preservative.

`Graintona Plus' (produced by FSL Bells Ltd.) was one of the liquid preservatives used in this experimental research. It was applied at the rate of 8l per tonne to the barley grain during the crimping process, irrespective of the moisture content. The two main functions of the non-corrosive `Graintona Plus' are; to increase the digestion period for cereals in the rumen and to prevent seed germination and the growth of yeast mould and bacteria in the container/ clamp/ silo, both during storage and when the face of the clamp is exposed after opening. The molasses constituent is an appetizer and improves palatability and digestibility of the barley grain.

The basic principles for the ensilation of cultivated high moisture barley are consistent with those of good grass silage making, in order to ensure a valuable source of `home grown' feed. Monitoring of the moisture content can take place at frequent intervals during the process of ensiling.

`Results of tests show that hermetically stored grain (barley and wheat) can be kept undried and in a mould free state for some weeks after removal from hermetic conditions, provided that the air temperature is reasonably low.'

[Hyde & Oxley, 1960]

Below zero temperatures do not appreciably affect ensiled cultivated barley with a high moisture content.

1.7.1 Advantages and Disadvantages of High Moisture Barley.

Advantages.

1. The harvesting of cultivated, high moisture barley can take place two to three weeks earlier than the traditional harvesting period.

2. Time is not lost waiting for green patches in the field to mature.
3. The harvest period is not dependent on climatic conditions and modern combine harvesters can operate during the wet weather periods.
4. Harvesting of the barley at high moisture levels results in an increase of around 20% in the dry matter yield of the grain, which is approximately 0.5 tonne more dry matter per acre (Krall, 1969; Kennelly et al., 1988b; Fletcher, 1988).
5. Early harvesting of the barley crops reduces contamination with weeds, especially wild oats, as the seeds are collected before shattering.
6. The dust levels at harvest are much reduced, decreasing the occurrence of 'farmer's lung'.
7. Threshing and shedding losses are lower.
8. The resultant feed from the high moisture, ensiled barley is very pleasant and moist, with a high palatability. It is extremely well accepted in feed rations by ruminants of all ages and it is highly digestible (Krall, 1969; Mathison et al., 1989; FSL Bells, 1989).
9. It is capable of replacing bought, commercial feeding products on a weight to weight basis, when sufficient good quality silage is available in the feed ration.
10. The straw value from a barley crop which has been cut early is significantly higher in terms of protein and digestibility.
11. Only small amounts of effluent are produced by an ensiled, high moisture barley crop.
12. It is possible for the user to remove small quantities of the grain from the container/ clamp/ silo at intervals as required.
13. Cattle can be directly self-fed from a clamp/ silo equipped

with a movable manager.

14. Through the completion of early harvesting, the land becomes available for the growth of a second crop, for example, rape or turnips. Alternatively, the greater length of time can be used for land and weed management.

Disadvantages.

1. The added moisture causes additional weight to the barley crop as it is harvested. 20% to 25% more weight must be carried.
2. The combine harvester has to progress at a slower speed because of the additional weight.
3. Unloading of the harvested high moisture barley must take place immediately to prevent 'caking', where as dry, mature grain may be left for several days in the combine harvester or container.
4. Barley grain with a high moisture content does not flow as freely as dry, mature barley.
5. At very high moisture levels or with extended periods of storage, it is possible for a certain amount of compaction to occur in the lowest part of the container, due to pressure on the relatively soft grain by the grain above. However, this temporary 'caking' is easily dispersed.
6. Mould contamination may occur, therefore airtightness is an absolute necessity to prevent mould growth on the moist grain (Hyde & Oxley, 1960).
7. At high moisture levels the intergranular air and the barley grain itself may develop a characteristic sour-sweet smell. The intensity of the smell may increase with the length of storage time, the moisture content and the temperature. If not too pronounced the smell is easily removed by airing.
8. Cultivated barley grain with a high moisture content will soon

heat and deteriorate if air is allowed to enter the silo; or if left in an untreated, or incorrectly treated state for a long period. In this condition the grain would be most unsatisfactory for nutrient utilization by animals (Mathison et al., 1989). If left long enough, it may cause spontaneous combustion and fire.

9. Problems of access to the container/ clamp/ silo may occur.

10. The high moisture content of cultivated barley may make marketing a problem, if the grain is to be sold commercially from the farm.

Good ensilation techniques of preserving, rapid filling and thorough packing and sealing of the high moisture barley, along with an adequate feeding rate, will provide a palatable, high quality meal which can be fed directly to ruminant animals, without further processing. Ensiled high moisture barley provides an exceptional source of 'home grown' feed from the conservation of a 'home grown' cereal.

1.7.2 The Comparative Feeding Value of High Moisture Barley.

The potential of ensiled, high moisture barley as a valuable feed source has been referred to earlier in the literature. In general, when cereal grains with a high moisture content such as barley, wheat, oats, maize or sorghum are fed to ruminant animals, pigs and poultry, they are at least equivalent to the respective dry mature grain when comparisons are made on a dry matter basis. The majority of studies have been conducted with dairy cows and cattle of various ages, as it is thought that sheep can utilize grain much more effectively than cattle (Orskov, 1976; 1979; 1986).

One of the chief advantages of barley with a high moisture content is that it provides a much more acceptable and palatable feed for all ruminants and the problems which are associated with

dry, mature barley in respect to cattle going 'off-feed' are not encountered with high moisture barley. According to Perry (1980) and Mathison et al. (1988), barley cereal with a high moisture content is much more acceptable in the feed ration to beef cattle than dry, mature barley.

Many researchers have found that daily gains and feeding efficiency of high moisture barley are comparable with that of dry, mature barley. Marx (1973) and MacLeod et al. (1973) reported equal milk and fat production of cows receiving 6.8 kg/d ensiled high moisture barley, or dry mature barley. The object of the research by Ingalls et al. (1974) was to compare the performance and certain physiological parameters of dairy cows fed diets containing barley at 14% moisture, compared to barley at 27% to 30% moisture either preserved, or ensiled. Their study would suggest that dry mature barley, or high moisture barley either ensiled, or treated with a 'Chemstore' acid preservative resulted in equal total dry matter and fat corrected milk. Whole barley with a high moisture content treated with sodium hydroxide, has been found to have a feeding value equal to that of dry mature barley (Orskov et al., 1981; Barnes & Orskov, 1982). Kennelly et al. (1988b) stated that the overall results of three experiments with a large number of steers indicated that, on a dry matter basis, high moisture barley had similar feeding value to dry mature barley and that there were no major differences between pit stored high moisture barley and 'Harvestore' silo high moisture barley. These researchers also stated that the substitution of high moisture barley for dry barley on a dry matter basis would promote a similar performance and carcass quality. Dinusson et al. (1964) found from their feeding trials that high moisture barley fed to weaned calves

resulted in a higher (0.98 vs. 0.93 kg/d) average daily gain, than dry mature barley. In a comparative feeding value trial using high moisture maize for dairy cows, Clark and Harshbarger (1972), using 50 cows in lactation, fed 6 diets for a period of 16 weeks and found that cows fed ensiled, high moisture maize and propionic acid treated maize produced more milk than cows fed dry, mature maize. Similar results had also been reported by Clark et al. (1973). In the feed trials of Kovin'ko et al. (1981) a 4.6% improvement was shown in gain by beef bulls fed high moisture barley compared to those fed dry, rolled mature barley. Laksessvela (1981) reported that pregnant ewes fed ammoniated, high moisture barley gained 18.9kg over an 18 week period, in comparison with a gain of 14.5kg and 11.6kg by ewes fed untreated or ground dry barley respectively. Mathison et al. (1989) stated from the results of their feed trials with 60 crossbred steers, that the steers fed barley with a 32% moisture content, treated with 1.1% and 2.3% ammonia, had overall daily gains which were 18% and 14% higher than those of the cattle fed the control dry barley. At the end of the 80 day trial, the cattle fed ammonia preserved 32% high moisture barley had heavier carcasses than those fed the control dry barley.

High moisture barley has definite possibilities as a feed grain for fattening cattle (Krall, 1969; Kennelly et al., 1988a,b; Fletcher, 1988; Mathison et al., 1989). The period of time required to bring cattle up to full feed and to reach a choice finishing grade is reduced by 10 to 15 days. This undoubtedly results in a number of economic advantages for the farmers and producers of both grain and cattle. When the grain is 'home grown' then this offers additional economic advantages to the producer and those involved in the dairying industry.

Cattle fed ensiled high moisture barley gained 8.6% faster and required 9.3% less feed per unit of gain than did cattle fed dry rolled barley (Perry, 1980). A higher finishing grade at the end of the experimental feeding trial has been observed by Krall (1969), Kennelly et al. (1988b), Mathison et al. (1989) and many other researchers. The use of ensiled high moisture barley in the feeding of ruminants has been advocated on the premise that barley in the high moisture state reduces the incidence of digestive upsets, especially those in cattle which are associated with the change over to a concentrate diet. It is purported that the rumen can adjust more rapidly to the intake of high moisture grain than dry mature barley (Krall, 1969; Perry, 1980). McKnight et al. (1973), state in their research that diets containing ensiled high moisture maize and acid-treated maize with a high moisture content proved to be more digestible in terms of dry matter, organic matter and energy than the diets containing dry, mature maize. The ensiled and acid-treated high moisture maize tended to have a slower rate of passage from the rumen and increased rumen digestion compared to dry maize, which could explain the overall digestibility of the high moisture maize. Similar results were obtained by McNeill et al. (1971) with ensiled, high moisture sorghum. All the researchers who used large numbers of cattle in their studies and feeding trials stated that the animals remained healthy throughout the trial period. In other research studies, the percentage of liver abscesses has appeared to be the same for high moisture grain feeding as for dry grain feeding. Palmquist and Conrad (1970) reported that rumen ammonia levels were depressed when high moisture barley was fed rather than dry barley. Laksesvela (1981) has shown that ammoniated high moisture barley was

digested more completely by lambs, than either whole barley grain or ground barley.

A number of researchers have found that the overall intake of roughage, silage or hay is reduced when high moisture grain is given as the main concentrate in the feed ration of ruminants. Dry matter intake was lower for cows receiving high moisture maize as a major source of energy in place of dry maize (McCaffree & Merrill, 1968; Palmquist, 1970). Dickey and Leonard (1973) found that high yielding cows in lactation consumed less dry matter and more protein on ensiled high moisture, shelled maize (with or without urea), than with the regular dry maize rations. Ingalls *et al.* (1974) stated that, in their conducted feeding trials, silage intake appeared to be lower for cows receiving the high moisture barley treated with propionic and acetic acid. However, Jones *et al.* (1970), Forsyth *et al.* (1972), Larsen *et al.* (1972) and other researchers have reported an equal intake of roughage with propionic acid treated maize, ensiled high moisture maize and dry maize feed rations. In their assessment of the feeding value of acid-treated high moisture maize (1.25% acetic and 1.00% propionic) for Holstein cows, MacLeod *et al.* (1974) found that voluntary forage intake was not reduced by the inclusion of high moisture maize as the concentrate ration. From their experiments with feedlot cattle some researchers claimed that feeding hay as the roughage source with high moisture barley as the concentrate in the ration produced higher dry matter intake and better performance than if silage was fed with the concentrates. Kennelly *et al.* (1988b) stated that roughage source (hay or cereal silage) was found to have no influence on the performance of 246 weaned calves, fed high moisture barley. At the present time much more research and

information is required to evaluate the influence of the roughage source used with high moisture grains, especially high moisture barley. When Flipot and Pelletier (1980), using Holstein steers, evaluated the feeding value of high moisture barley against dry barley grain, they observed no significant differences in dry matter intake, average daily gain, live weight gain or carcass characteristics. It has been found that the relative particle size of high moisture barley (and dry barley) after processing could have an important modifying effect on the relative rates of rumen degradation and that feeding whole unprocessed high moisture grain, especially to cattle, is not a satisfactory procedure. Recent research in Finland (Fletcher, 1988), has shown that with the use of an ensiled, high moisture mixed crop of barley, oats and peas (plus added minerals and vitamin E), an average milk yield of 7400kg per cow has been obtained in a Finnish Ayrshire herd.

It could be concluded, that the different final results obtained by the many researchers may be a reflection of the source of cereal genetic variety, the processing method and the ensilation conditions.

1.8 CONDITIONS FOR THE CULTIVATION OF BARLEY.

The barley plant has a broad ecological adaptation that sets it apart from other cereals and gives it a distinct advantage.

Climate.

Barley is a cereal crop which grows well in the temperate climates and thrives best where the seasons are cool and moderately dry. Barley is grown in a wide range of photoperiods and it is a quantitative long day species, with day length sensitivity differing between the genetic varieties. It has been

found that barley ripens earlier than other cereals. This suggests that barley requires few heat units to reach physiological maturity (Rasmusson, 1985). The barley crop may be injured by frosts during the flowering and early grain filling periods.

Soil conditions.

Cultivated barley grows best on a well drained, fertile loam, or light, clay soil. Heavy, clay soils may be undesirable for the production of barley, if they become too wet or waterlogged. It is possible to obtain extremely high yields on clay soil, if there is a good tillage and soil moisture. Barley seed is sown at a depth of 2cm to 6cm and deep seeding has proved to be detrimental to a crop. Barley is more tolerant than other cereals to alkaline soils and less tolerant to acid soils. A soil pH range of 6.0 to 8.5 is generally acceptable for barley plant growth. Barley is often regarded as being a drought resistant crop.

Length of accessibility period.

The amount of time during the year that the land can be worked by machinery (or grazed by animals), controls to a large extent the pattern of farming practice that is possible in any geographical area. The number of days between the end of field capacity in the Spring and the return to field capacity (of moisture) in the Autumn, provides a good approximation to the accessibility period of the land, for example, early March to early October for Ayrshire, Scotland (Francis, 1981).

Barley is cultivated in certain geographical areas for specific reasons, but it has been found that, overall, two-rowed barley is preferred to the six-rowed variety for the following reasons:

1. It is more shatter resistant.
2. It is combine harvested and cleaned more easily.
3. The large seeds are preferred for feed grain.
4. It is preferred by the manufacturers and export markets.

The ultimate yield of the barley crop is dependent upon the resistance of the genetic variety and the interaction between the weather and the soil factors (Russell, 1988).

1.8.1 Moulds and Fungi Important to Stored Barley Grain.

Every year a considerable amount of all harvested grain has to be discarded because of deterioration due to mould. Moulds will develop in stored grain when the moisture content exceeds 18% to 20% and the ambient temperature is sufficiently high. The moisture content of the grain and the temperature basically reflect the degree of deterioration, as do the the harvesting conditions and storage. Moulds will never grow on grain which has a moisture content of less than 12% to 13%. The limits for species such as *Aspergillus* average 16% to 19%. Fungi are aerobic and their growth is retarded by 10% carbon dioxide and strongly checked by 20% carbon dioxide (Briggs, 1978). Several species of moulds which grow on fields, or stored grains are able to produce mycotoxins (Smith et al., 1984). Chemical analysis has demonstrated the presence of different potential mycotoxins in animal feeds of good quality, as well as those of unacceptable hygienic quality (Stenwig & Liven, 1988). Fungi are common throughout nature and changes may occur in stored grain before the mould becomes visible to the naked eye. In most cases of natural contamination, more than one species of mould is usually involved and they are usually in different growth stages.

Three important factors are responsible for mould development. They are:

1. The moisture content of the grain.
2. The ambient temperature: most rapid growth occurs at 30°C to 32°C.
3. Length of storage time.

Major alterations to stored barley attributed to fungal invasion:

1. Decreased germinability.
2. Discolouration of the embryo or entire kernel.
3. Heating and mustiness.
4. Potential production of harmful toxins.
5. Biochemical changes within the grain, affecting the availability of micronutrients and feeding value.
6. Loss of palatability.
7. Loss of weight.

In cultivated barley varieties, most of the micro-organisms are found in the hull (husk), particularly between the hull (husk) and the pericarp and also in the pericarp itself.

Types of fungi important to grain storage.

The fungi genera of greatest concern are *Penicillium*, *Fusarium* and *Aspergillus*, because these include important toxigenic species.

1. *Penicillia*.

These are the most common of the moulds and up to sixteen species have been found in stored cereal grain samples. Examples include: *P. funiculosum*, *P. hordei* (the most frequent species in Danish stored barley), *P. cyclopium*, *P. expansum* and *P. roquefortii* (the most frequent species in UK stored barley grain). *P. puberulum*, *P. brevis compactum*, *P. viridicatum* and *P. melanochlorum* are the four most common species found in Norwegian stored barley (Stenwig & Liven, 1988). These four common species have also been found in

stored barley in the UK (Hill & Lacey, 1984). *Penicillium* mould has been related to the haemorrhagic syndrome in poultry.

2. *Fusarium*.

Twelve species have been found to occur in stored grain. These moulds are more common in stored barley grain than in stored oats. They are the most common cause of blight and decay and can be found in cribbed grain, especially in Central and Western USA (Jones et al., 1974). *Fusarium* has been associated with the oestrogenic syndrome in ruminants and pigs, giving enlarged uteri, reduced litters and abortions.

3. *Aspergillus*.

A 3% to 5% invasion of *A. flavus* on stored barley would result in a potentially toxic feed. If the grain is fed to lactating cows, the toxins are secreted into the milk as Aflatoxin M₁. Aflatoxin B₁ is one of the most carcinogenic agents known (Jones et al., 1974). Ochratoxin A is also a natural contaminant of mouldy, stored grain.

Effects of aflatoxins on animal performance.

Feed contamination results in loss of condition and poor performance in livestock. Lung lesions and pneumonia are common. Cows and heifers soon lose condition, suffer liver damage and there is also a marked reduction in lactation when they have eaten grain affected by mould. Much of the aflatoxins are retained in the body of the animal, especially in the liver and this retards the recovery of a ruminant animal. Calves below six months of age are highly susceptible to aflatoxicosis.

Rotter et al. (1989a) found the species *P. cyclopium* and *A. flavus* in contaminated barley samples. They suggested that the nutrients in the mould-contaminated barley were less available and also, that digestion or uptake of the dietary nutrients from

all components are subjected to interference. Knowledge of the fungal content of barley grain aids considerably in the formulation of feed and therefore reduces costly losses, both of grain and animals. The degree of fungal contamination of grain or feed sample can be estimated from the determination of its glucosamine content, using a modified amino acid procedure (Rotter *et al.*, 1989b). Identification of the presence of a toxin does not provide positive evidence of toxin production within the grain. Feed grain contaminated by mould may also affect the end product, especially meat and milk for the consumer, when it is fed at levels that are toxic to ruminant animals. The importance of the proper storage of cultivated barley (and all grains) for all animals cannot be overemphasized.

1.8.2 Additives for High Moisture Barley.

Successful ensilation is dependent upon the control of spoilage by aerobic organisms. Anaerobic conditions within a silo must be achieved rapidly and subsequently maintained. Continued preservation depends upon the rapid lowering of pH, to prevent degradation by enterobacteria and clostridial bacteria. To achieve anaerobic conditions, meticulous attention to detail must be observed throughout the whole ensilation process. Under natural conditions, the lowering of pH is dependent upon the fermentation of the sugars within the crop to lactic acid, by the bacteria present in the microflora of the crop, the prominent being *L. curvatus* and *L. plantarum*.

The Embden-Meyerhof-Parnas glycolytic pathway is the mechanism involved in the initial stages of glucose fermentation by the homolactic organisms. The two pyruvate molecules formed from either glucose or fructose are subsequently reduced to two moles of lactic acid (McDonald, 1981). Lactic acid bacteria are

facultative anaerobes and consequently, they possess active aerobic pathways, using oxygen as a terminal hydrogen acceptor, which combines with atoms of hydrogen to form hydrogen peroxide. The products of the aerobic metabolism of glucose and fructose by the homolactic bacteria include lactate, acetate, formate, acetoin, water and carbon dioxide. A number of lactic acid bacteria are able to ferment pentose sugars. Xylose and arabinose may become available as substrates from the hydrolysis of plant hemicelluloses and these are fermented to a mixture of lactic and acetic acids.

The active ingredients in additives can be classified into four main categories (Figure 1.5):

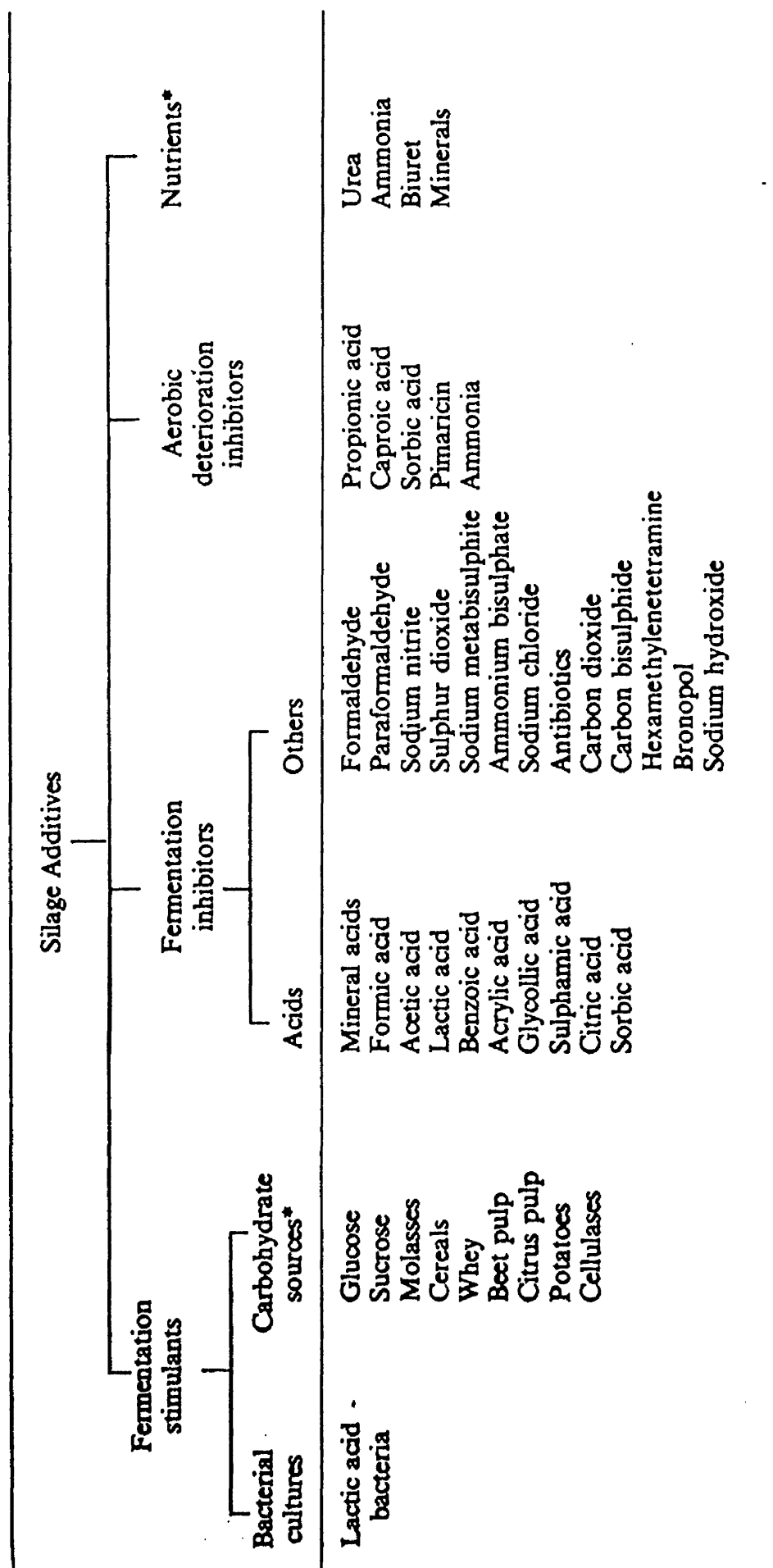
1. Fermentation stimulants.
2. Fermentation inhibitors.
3. Aerobic deterioration inhibitors.
4. Nutrients.

1. Fermentation stimulants.

The main purpose of these is to encourage lactic acid fermentation and they are of three main types:

- a) *Innoculants*. These are bacterial cultures which supply the naturally occurring homolactic bacteria.
- b) *Carbohydrate-rich materials*. These are added to increase the supply of available energy for the homolactic bacteria. Molasses, sugars, beetroot pulp and citrus pulp have been used for this purpose.
- c) *Enzymes*. Cellulolytic enzyme fermentation results in the breakdown of the cellulose and the hemicellulose to improve the digestibility of the organic matter and increase the content of fermentable sugars available to the homolactic bacteria.

Figure 1.5 Classification of silage additives



* Most substances listed under carbohydrate sources can also be listed under nutrients

Taken from McDONALD (1981).

2. Fermentation inhibitors.

These may be acidifiers or sterilants. Acidifiers produce quantitative changes in the microflora of the ensiled crop and lower the pH rapidly, therefore stimulating the lactic acid bacteria. They are organic acids such as formic acid and acid salts. Formic acid, commercially known as Formalin is not used on its own, but as a major or minor constituent in acid/ formalin mixtures. The mineral acids, for example, sulphuric mixtures and phosphoric acid are also used. Sodium nitrate is another sterilant sometimes used.

3. Aerobic deterioration inhibitors.

These are primarily concerned with controlling the deterioration of the crop on exposure to air. The major organisms responsible for the aerobic deterioration of an ensiled crop include yeasts, bacteria and moulds. These have been discussed earlier (Section 1.8.1) and they are mainly indigenous to the ensiled crop and not aerial born invaders. Propionic acid has been widely used as a microbial inhibitor in the preservation of stored grains, (Jones et al., 1974) and also sodium propionate, sorbic acid and ammonia (McDonald, 1981). A number of additives have been laboratory tested as potential deterioration inhibitors, but very few have been subjected to use in feeding trials with animals.

4. Nutrients.

When these substances are added to the ensiled (natural) material, they make a significant contribution to the nutrient needs of the animals consuming the crop. Many of the additives listed under 1. above such as molasses, cereals, whey, beet pulp and citrus pulp may also be included along with urea, ammonia, biuret and minerals.

Absorbents.

These are mixed and layered into the crop at ensiling to absorb the effluent. The recommended rate of application is dependent on the dry matter content of the crop. It has been suggested that some of the products available are capable of absorbing up to six times their own weight of effluent (McDonald, 1981).

Chemical additives are effective in both the preservation and enhancing of high moisture grain. Maximum benefit is obtained if the additive is applied immediately after harvesting. A number of research studies have been carried out to obtain data on the efficacy of these additives as grain preservatives and on their effects on the performance of animals. Formaldehyde (commercially available as Formalin) is well known as a sterilizing agent and for its important reaction with protein (Offer et al., 1971). Although formic acid is the strongest of the fatty acid series, it is considerably weaker than the minerals used in the A.I.V. process (Section 1.1), (McDonald, 1981).

The reaction of formaldehyde with protein.

In certain situations, the requirements of some individual ruminant animals cannot be met satisfactorily from the supply of feed from normal sources. Therefore, the need to modify the extent to which the protein in the diet is degraded in the rumen, that is, 'protect' the protein, has led to the development of a number of techniques. One such technique is the use of formaldehyde chemical treatment. Formaldehyde has been used extensively to increase the resistance of dietary protein and starch to microbial digestion in the rumen (McAllister, 1990a). In the reaction between formaldehyde and protein,

the initial step appears to be the rapid formation of a methylol compound.... Condensation reactions then take place slowly over time, with the formation of stable methylene cross - linkages between protein chains. These

reactions of HCHO with the amino acid groupings take place under differing conditions of pH and temperature.'
[Barry, 1976]

By increasing the resistance of dietary protein to microbial degradation in the rumen, formaldehyde treatment has been shown to increase the supply of amino acids to the intestines, resulting in an increase in protein digestion and nitrogen retention (Offer et al., 1971; McDonald & Warner, 1975; Barry, 1976). Research studies using formaldehyde treatment for the protection of dietary protein have been presented by: Offer et al. (1971), Hughes et al. (1971), Faichney & White (1977a,b), Lindsay et al. (1983) Kassem et al. (1987), Ravelo et al. (1988), Martin & Thomas (1988), Van Ramshorst & Thomas (1988), Fluharty & Loerch (1989), Morgan et al. (1989), Hyslop et al. (1989) and, most recently, by McAllister et al. (1990a).

Studies at the Hannah Research Institute (1986), involving the formaldehyde treatment of barley at rates of 8 to 15 l/tonne, gave notable effects on the rates of digestion of barley protein and starch. Over a 24 hour period of rumen incubation, the protein degradability of untreated samples was 70% to 80%, compared with values of 20% to 50% for formaldehyde-treated barley. The corresponding rates for starch were 90% to 98% (untreated) and 70% to 80% (treated). Investigations with sheep given dried grass and untreated or treated barley (60:40 DM), showed that the treated grain had little effect on rumen fermentation pattern but had significant influences on the passage of nutrients from the rumen to the small intestine. With lactating cows, the milk yield effects observed were partly due to a modification in the cow's use of nutrients for milk secretion. Formaldehyde-treated barley gave increases in protein and lactose yields which were much greater than those for fat

yield. In some instances, the effects on fat yield were negative. An average increase in milk yield of 4.7% was obtained with the formaldehyde-treated barley. With good quality silages, responses of approximately 7% to 8% above the control yields were obtained.

Kassem et al. (1987) found significant increases in milk production and silage intake with barley treated with formaldehyde at a rate of 15 l/tonne. There were tendencies for formaldehyde-treated barley to be associated with an increase in milk protein content and a slightly decreased milk fat content. They suggested that the findings could not be fully explained by the increase in silage dry matter intake and that the chemical treatment of the barley supplement may have modified the supply of nutrients to the cow's tissues, improving the digestibility of the diet or increasing starch and protein flow to the small intestine. Martin and Thomas (1988) also found formaldehyde treatment of barley tended to increase milk yield and reduce milk fat content. However, the milk fat composition and the yields of individual fatty acids in milk showed no significant change from untreated barley. They suggested that the protein matrix of formaldehyde-treated barley would protect the cereal lipid against ruminal biohydrogenation by creating a physical barrier between the lipid and the rumen micro-organisms. This protection against microbial biohydrogenation was not found, despite indications that the protein and starch components showed reduced ruminal degradation.

In sheep given diets of dried grass and rolled barley (60:40 DM), no significant differences in total or individual volatile fatty acids, rumen pH, protozoal numbers and concentrations of ammonia nitrogen were seen between formaldehyde-treated barley and an untreated control (Van Ramshorst & Thomas, 1988).

Formaldehyde-treated barley increased duodenal passage of total nitrogen by 3g/ day and doubled the duodenal passage of starch. There were no significant differences between treatments in the digestion of acid detergent fibre, or in organic matter digestibility between the two diets. For the treated diet, it was found that the reduced proportion of organic matter digested in the stomach was compensated for by an increase in OM digested in the lower gastro-intestinal tract, as the duodenal flow of OM for the treated barley was significantly greater than for the untreated diet. However, the sheep in this investigation were fed at hourly intervals throughout the whole day, which was not representative of production conditions. Also in investigations with sheep, Hyslop *et al.* (1989), found a 14% increase in hay intake with mature wether sheep fed formaldehyde-treated barley compared to untreated barley. The rumen pH of the sheep fed the control barley diet fell to a minimum of 6.0 - the 'cellulolysis threshold' (Mould & Orskov, 1983), whereas for those fed the treated barley the minimum pH recorded was 6.25. With a forage to concentrate ratio of 70:30 (DM), a shift in the acetate: propionate ratio was not observed. Hyslop *et al.* (1987), anticipated a positive production response to formaldehyde treatment of barley as there was no significant change in diet dry matter digestibility . They also suggested that the reduced ruminal digestion of barley was balanced by increased digestion of hay in the rumen and a shift in the site of barley digestion to the intestines.

McAllister *et al.* (1990a) confirmed the greater effect of formaldehyde treatment on barley protein degradability seen by other researchers (Kassem *et al.*, 1987; Morgan, 1989), by presenting visual evidence (using scanning electron microscopy)

which showed that formaldehyde maintains the integrity of the protein matrix against rumen microbial degradation. This matrix inhibits the access of rumen microbes to underlying starch granules, increasing the lag time of starch fermentation. As Kassem *et al.* (1987) conclude;

'in cows given silage *ad libitum* with supplementary concentrates containing barley, worthwhile benefits in milk production can be obtained through chemical treatment of the barley to reduce the rate of degradation of starch and protein in the rumen.'

Other additives.

Propionic acid is the most effective antimycotic agent of the lower fatty acids. It has a beneficial effect in controlling silage fermentation and has been found to reduce ammonia-nitrogen formation in ensiled crops; to stimulate the growth of lactic acid bacteria and to improve silage dry matter intake (McDonald, 1981). The work of a number of researchers and data involving the use of propionic acid, acetic acid, acetic-propionic-butyric acid and sodium propionate, has been presented by Jones *et al.* (1974) in their review. Ammonia has also been used as an additive in the ensilation of high moisture barley. In the research trials conducted by Mathison *et al.* (1989), high moisture barley appeared to be well preserved, but results from other trials have been variable. Rode *et al.* (1986) and Mandell *et al.* (1988), confirmed that ammoniated whole barley was degraded more slowly in the rumen than the control barley grain. Data of Robinson & Kennelly (1988a,b), using rolled, high moisture barley also demonstrated this. Levels of 1.5% and 2% ammonium isobutyrate effectively preserved high moisture grain. A decrease in methane production with ammoniated, high moisture barley has been reported by Delfino *et al.* (1988). Commercial additives containing ammonia also frequently contain minerals and

molasses, the latter ensures that additional fermentation acids are provided in order to offset the increased buffering effect of ammonia (McDonald, 1981). Other additives which have been used with high moisture barley and other high moisture grains in research trials are: sulphur dioxide (Gibson et al., 1988; Mathison et al., 1988; 1989), sodium hydroxide, calcium/ sodium nitrite, sodium metabisulphite (Mathison et al., 1988), sodium chloride (Orskov et al., 1971) and ammonium tetraformate.

Desirable characteristics of grain preservatives.

Grain preservatives are evaluated as to mould growth, temperature rise, weight loss of stored grain and feeding studies. The desirable characteristics should comprise of compounds that:

1. Are metabolized by a known pathway.
2. Exhibit a very low order of mammalian toxicity.
3. Possess no mutagenic, carcinogenic or teratogenic properties.
4. Do not adversely affect the nutrient value of the grain.
5. Leave no residue in the animal tissue or consumer product.

1.9 POSSIBLE FINDINGS FROM THE RESEARCH.

From the literature, it can be seen that over the years, a number of hypotheses and theories pertaining to grain of a high moisture content have been postulated by research scientists from different countries.

In this experimental scientific research on cultivated High Moisture Barley (genetic variety Atem), a number of factors will be considered. The complete ensilation process will be investigated and the effectiveness of additives will be evaluated. *In vivo* degradability studies will be made and a feed trial will be conducted to discover the potential feeding value of this barley and its effect on the performance of ruminant

animals. Knowledge in all fields is continually expanding through research. This study will be concerned with finding some of the scientifically accepted answers to the many questions involved and will also endeavour to find new methods to utilize more efficiently the cereal grain that is available for animals. It is hoped that some interesting observations will be made and relationships discovered which are worthy of consideration; also that the information recorded and the results and data which will be assembled will at least substantiate some of the theoretical findings and results which have been obtained by other scientific researchers on the subject of High Moisture Barley.

PART TWO.

THE EXPERIMENTAL STUDY.

2.1 The Objective of this Research Study.

This research study was undertaken in three main experimental areas :

Experiment 1 was concerned with the ensilage, storage and chemical characteristics of the barley grain. Investigations into the conditions required for the formation of a stable product were undertaken. The keeping value, the stability in storage and the stability to air were assessed, as were the amount and type of fermentation acids produced.

Experiments 2 and 3 centred on *in vivo* investigations on the grain.

Experiment 2 was based on the bag technique of Mehrez and Orskov (1977). The degradability of nitrogen, organic matter and protein - free organic matter was assessed for five major barley treatments.

Experiment 3. A feeding trial was conducted using ten sheep. Five crimped grain treatments and one of untreated grain were fed according to an incomplete block design consisting of three, 20 day periods.

All three experiments were carried out at the West of Scotland College, experiments 2 and 3 being performed at the purposely designed Metabolism Unit.

2.2 Collection of the Grain.

In the early harvest period of August 1989, approximately 1.6 tonnes of moist barley at a dry matter content of 549 g/kg were obtained from a farm in the Dumfries and Galloway region of SW Scotland. Atem was the variety of barley used and at harvest a number of the grains had a green appearance. On the farm, the grain was crimped (bruised, kibbled or cracked) and treatments simultaneously applied by means of a MURSKA 350S crimping machine. This machine (marketed by FSL Bells Ltd.) is especially designed with pitted rollers for the crimping and addition of treatments to moist grain.

Five 250kg batches of moist barley grain were obtained and to each was applied one of the following liquid treatments at the application rate stated:

Table 2.1 Major treatments.

100% Graintona Plus	8	l / tonne.
50% Graintona Plus	8	l / tonne.
100% Sylade 2	8	l / tonne.
50% Sylade 2	8	l / tonne.
Cane Molasses	6.4	l / tonne.

In addition, a 250kg batch of grain was crimped, but no treatment was applied. This was obtained for control purposes and to conduct smaller scale experiments which would be used to investigate the effect of additional treatments on ensilage (Experiment 1).

Care was taken to ensure an accurate treatment application rate by means of the flowmeter attached to the 'Graintona Plus' treatment store. For application purposes, the Molasses was diluted, using distilled water, to 60% of its original consistency but this still proved too viscous to ensure an

accurate flow rate at 8l/tonne. Therefore, a rate of 10.6 l/tonne of the 60% solution had to be used, as this was found to be the most constant for such a viscous material. This was equivalent to an application rate of 6.4 l/tonne of pure Cane Molasses.

After each application, an amount of grain was run through the crimping machine but not collected, to ensure that no cross contamination of treatments arose.

'Graintona Plus' (FSL Bells Ltd.), based on formaldehyde, is especially designed for the treatment of moist grain and is applied at the rate of 8l/ tonne irrespective of the grain moisture content. 'Sylade 2' (ICI plc) is an acid/ formalin based additive designed for silage treatment. In this experiment, it was applied at a slightly higher rate than recommended for silage (2.5 - 5l/t) to be used as a direct comparison with the Graintona treatment. The recommended application rate for Cane Molasses (Intermol), when used for silage, is between 4.5 and 18l/ tonne.

To make up the half strength (50%) treatments, a known volume of the original liquid was simply diluted with an equal volume of distilled water. The five prepared treatments, plus the control grain, were designated **MAJOR** treatments, the majority of each to be used in a feed trial (Experiment 3). The six batches of grain were collected in plastic sacks (7/8 per batch), tied and transported to the West of Scotland College Metabolism Unit. The day of crimping and collection of the grain was designated Day "0" - the baseline for subsequent readings.

2.3 Experiment 1: Preservation studies.

On the following day (Day 1) each of the five major treatments obtained was ensiled in three different sizes of container.

a) *Large barrels (150 kg)*. These were constructed of heavy duty plastic and had tight fitting, screw top lids. They were lined

with a heavy gauge polythene bag to act as an airtight seal for the grain. The barrels were filled gradually by pouring grain from the appropriate plastic sack, a little at a time and trampling it down thoroughly to exclude as much air as possible. When the grain level reached the rim of the barrel, the plastic liner was gathered up around the gauze-covered hose of an industrial vacuum cleaner (Numatic 1900W), which was used to evacuate any remaining air from the contents. The neck of the liner was twisted, double tied, folded and retied to ensure maximum air exclusion. Finally, the lid was fitted and the screw top replaced tightly. Two large barrels were filled with each of the five major treatments, by repeating the above process. Untreated grain was not prepared using this method to avoid the possibility of large amounts of spoilage. The barrels were then left in an outside shed to ensile.

b) *Pot silos (4kg)*. Constructed of plastic, with a tight fitting lid, three of these containers were used for each of the major treatments to give triplicated results. A plastic bag of suitable size and strength was filled with barley grain, placed in the plastic pot and compressed to fit. Each pot was then filled level to the rim with grain, further compressed and a neck made from the liner. Whilst being compressed the bag liner was double tied, folded and tied again to exclude the maximum possible amount of air. Spaces between the plastic bag and walls of the pot silo were filled with sawdust, as was the space between the top of the tied liner and the lid. The pot silo lid was then clipped on, any excess sawdust being removed in the process. This procedure was used to prepare, in triplicate, pot silos for the major treatments (5 treatments + control). The eighteen pot silos prepared were then left with the large barrels in the outside

shed to ensile.

c) *pH pots* (0.3kg). These were actually cylindrical 'Tupperware' airtight containers identical to those for domestic food use. No inner liner was used for these small pots. Five small pots were filled with grain from each of the major treatments and with untreated grain. The grain in the pots was tightly packed and each pot was filled to the brim. Excess grain was then brushed from the brim and the airtight lid carefully applied, ensuring no grains were trapped between the seal of the lid and brim. One transparent pot for each of the five treatments was used in order that any mould growth might be observed, this would be opened last. All pots used for the untreated grain were transparent. These containers were so named as they would be opened at selected intervals throughout the ensiling process to monitor pH and storage characteristics. The small pots were then left with the pot silos and large barrels to ensile.

At the end of Day 1, four plastic sacks of untreated barley grain were placed in the walk-in freezer (-20°C) and three plastic sacks placed in the fridge (0°C). The former sacks were frozen for long term storage for use in the feeding trial, the latter sacks were merely kept cool in readiness for preparation of the minor treatments.

During the next two days, seven further treatments, designated as **MINOR** treatments were prepared from the cooled, untreated grain (Table 2.2). A 15kg batch of this grain was obtained and allowed to reach ambient air temperature, so ensuring that none of the grain contained ice crystals or frozen clumps.

Table 2.2 Minor treatments.

Cane Molasses 12.6 l / tonne.

Cane Molasses 6.4 l / tonne + Forager Innoculant 2.5 l/tonne.

Cane Molasses 12.6 l / tonne + Forager Innoculant 2.5 l/tonne.

Forager

Innoculant 2.5 l / tonne.

Add - Safe 4.0 l / tonne.

Add - Safe 8.0 l / tonne.

Amylase 8.0 l / tonne of a solution containing 7.2×10^5

Enzyme Units per litre.

The grain was then placed in an electric cement mixer (Belle Minimix Mk.3) and mixed for three minutes to ensure uniformity. The chosen treatment, prepared for 15kg at an amount equivalent to the above application rates, was applied to the grain as a continuous spray, by means of a hand-operated trigger sprayer (Momar Manufacturing Chemists), whilst the mixer was in motion. This took approximately two minutes. The mixing was continued for a further four minutes to ensure even treatment distribution amongst the grain particles. The cement mixer was cleaned between dissimilar treatments to ensure no cross contamination occurred.

Forager silage inoculant (Shell Chemicals), made up from a powder according to the manufacturer's instructions, was applied at the recommended rate to the grain alone and to two treatment levels of molasses. The recommended rate for Add-Safe (B.P. Nutrition, UK), 4l/t, was used for one minor treatment and a higher rate, 8l/t, equal to that of the major treatments, was used for a second minor treatment preparation. α -amylase (BDH Chemicals Ltd.) was also applied at the major treatment rate to allow comparisons to be made. This treatment was designed to release the carbohydrate store from the barley grain, to be

fermented by the resident bacteria, in a similar way to the molasses treatment. Each of the minor treatments was ensiled in triplicate 4kg pot silos by exactly the same method described in the section above. They were then stored with all the other containers and left to ensile.

When all the treatments had been ensiled in all of their designated containers, some excess grain from the minor treatments was fed to sheep and goats on maintenance rations. The remaining half sacks of feed left over from the major treatments were tied and later used for pH sampling. These were kept until visible moulding was observed. On Day 14, significant moulding in these half sacks was evident and all but one were disposed of. The half sack of control grain was retained and allowed to mould for future observation and possible tests.

It was known that the pH of the grain would give an important insight into the ensiling process. Therefore it was recorded for the six feeds during their ensilation. Opening the barrels or pot silos would have destroyed the anaerobic atmosphere around the grains which, it was hoped, had been successfully achieved. Thus use was made of the small pH pots. The contents of these could be easily measured and then disposed of, allowing the larger ensilations to progress undisturbed. Readings of pH for Day 2 and Day 7 were made from the half-filled sacks mentioned earlier. The sack was untied and two amounts of grain, in excess of requirements, were obtained in two different areas, from well below the surface. The pH was measured as detailed in Section 2.4. On Day 14, it was decided the grain was too mouldy to give a fair and accurate result. On this day, a pH pot for each of the six feeds was obtained. Each pot was, in turn, opened and the top layer of grain (5cm), which was most

likely to have been contaminated by air seepage, was removed and discarded. Later in the ensilage process, this top layer became very dry and formed a mouldy, solid 'plug' which was easily removed, leaving satisfactory grain underneath. The pH was then measured as detailed (Section 2.4). Careful note was also taken of the appearance, smell and consistency of the barley grain. pH pots were opened at weekly intervals but as the difference between pH readings became less for each treatment, a greater time interval between openings was allowed to obtain a longer pH/ time profile from the given number of pots. On the days pH readings were taken, the pot silos and remaining pH pots were also examined to ensure that their lids were secure and the airtight seal was still intact.

On Day 97, the first sets of pot silos were opened. After removing the inner plastic bag from the silo, the string tie and adhering sawdust were removed. The bag was opened and weighed on a balance tared for the weight of a similar plastic bag. The weight was recorded, the contents emptied out and spread onto a large plastic tray. The wastage was assessed according to the method in Section 2.4. This procedure was repeated for the two remaining pot silos containing the treatment. The contents of the plastic tray were then thoroughly mixed and subsamples taken for pH and dry matter determinations and water extract analysis, as detailed in Section 2.4. The bulked contents of the three pot silos for each treatment were then replaced in the plastic bags. These were then placed untied in the pot silos and frozen at -20°C . The subsamples for water extract analysis were also frozen until they could be prepared. The above procedures and analyses were repeated for every treatment contained in the three pot silos until all the treatments were frozen by Day 118. Once the

dry matter had been determined on each treatment, the dried grain was milled (C & N Junior Laboratory Mill) through a 2mm screen and retained for nitrogen and organic matter analysis.

Aerobic stability.

An experiment was designed in order to obtain a more quantitative assessment of the stability to air or 'shelf life' of the six grain treatments. The grain used was obtained fresh from the large barrels whilst the feeding trial (Experiment 3) was under way. The control grain was obtained from a well mixed sample of previously thawed out feed which was being used in the trial. Three long (30 cm) glass, nitrogen digest tubes (diameter 3 cm) were obtained. Into each was poured 75g of barley grain from one treatment. The grain was not compressed but the tube was tapped whilst being filled in order to obtain a uniform grain content along the tube length. A further three tubes were prepared for each of the remaining treatments. The eighteen tubes were stored in racks in a shaded corner of a laboratory (average temperature 65°C) and allowed to mould. Periodic measurements of the extent of travel of both white and blue mould down the tubes were made. The extent of mould travel was expressed as a percentage of the whole tube length. Graphs were constructed to show the extent of moulding with time.

Buffering capacity.

As the barley grain was ensiled in a similar way to forage plants, it was for informative reasons that the buffering capacity of the crimped, untreated grain should be determined.

10g of previously thawed, well mixed grain was macerated with 250ml of chilled, distilled water for 2 minutes. The macerate was first acidified to remove any bicarbonate which, if present, would act as a buffer. The macerate was titrated to pH 3

with 0.1M hydrochloric acid and then titrated to pH 6 with 0.1M sodium hydroxide. The volume of standard alkali required between pH 4 and 6 was recorded. The buffering capacity was expressed as the number of milli-equivalents of alkali required to change the pH from 4 to 6 per kg dry matter.

2.4 Analytical Methods.

a) *pH*. A 10g sample of grain was weighed into a clean beaker, into which was poured 50 ml of distilled water. A magnetic flea was added and the mixture stirred briskly on a magnetic stirrer (Corning Hot-Plate Stirrer PC 351) for five minutes. After standing for a further minute, the mixture was agitated and the pH read using a previously calibrated, digital pH meter (Cranwell CR99). This procedure was repeated for a further 10g sample of grain, near the base of the pH pot or from another site in the case of larger amounts of grain and the mean pH for that treatment recorded. The above protocol was used whenever it was wished to determine the pH of a grain sample.

b) *Amount of moulding*. The amount of waste, mouldy grain from each pot silo was assessed visually and removed, in a similar way to that by a farmer. The amount of mould was weighed and expressed as a percentage of the total weight of grain contained in the pot silo. A mean value from the three pot silos for each treatment was then calculated and recorded to give an indication of the amount of waste resulting from each treatment.

c) *Dry matter (DM)*. Three representative samples of the grain (each approximately 150g) were spread evenly into metal foil trays of known weight. These were dried in a recirculating air oven at 60°C for 48 hours, cooled and reweighed. The DM content was calculated from the division of grain weight after oven drying by the weight before oven drying, multiplied by 1000 and

expressed in g/kg. In mathematical terms:

$$\text{DM (g/kg)} = \frac{\text{Dry weight}}{\text{Wet weight}} \times 1000.$$

The DM of Dacron bag residues was determined by drying the bag with its contents at 60°C for 48 hours in a recirculating air oven. After cooling in a desiccator, one bag at a time was taken, the string removed, and the bag weighed. The known weight of the empty bag was subtracted to give the DM weight of the bag contents after rumen incubation. The DM digested in the rumen was then calculated, knowing the initial DM weight of the bag contents and the results found as above.

d) *Organic matter (OM)*. Determined for feeds, faeces, refusals and Dacron bag residues by weighing approximately 2g of dry, milled material into a pre-weighed silica crucible. The contents were then ashed at 525°C in a muffle furnace (Gallenkamp) for 24 hours. After cooling in a desiccator and reweighing, the division of the weight of the crucible contents after ashing, by the weight before ashing gave (1-OM). The OM was then multiplied by 1000 to be expressed in g/kg DM. Mathematically:

$$\frac{\text{Ashed weight of crucible contents}}{\text{Dry weight of crucible contents}} = (1 - A) .$$

$$\text{Organic matter content (g/kg DM)} = A \times 1000.$$

OM was determined in triplicate, except for the case of Dacron bag residues where there was insufficient sample. OM for these residues was determined in duplicate and extra care was taken to zero the balance between weighings.

e) *Nitrogen determinations*. Total nitrogen in the feed weighed into Dacron bags and in the bag residues after incubation was determined using the standard Kjeldahl technique. An acidified selenium catalyst was added to approximately 0.25g DM of each

sample in duplicate. Addition of hydrogen peroxide initiated the digestion process. The samples were digested on a Tecator Block Digestion System 40, according to a pre-set programme. After cooling and dilution, a small sample of the acid digest (0.02ml) was taken for colourimetric determination of ammonia using the indophenol blue method.

f) *Protein - free organic matter determinations (PFOM)*. PFOM in the feeds and Dacron bag residues was determined by a simple arithmetical calculation, where PFOM is equal to total organic matter minus the crude protein of the sample ($PFOM = OM - CP$). Crude protein is simply nitrogen content multiplied by 6.25.

g) *Water extract analysis*. 40g of barley grain, which had been previously thawed overnight, was placed in a 250ml wide-necked bottle and 100ml of distilled water was added. The bottle was then capped and shaken on a 'Stuart Mechanical Flask Shaker' for 1 hour. The resulting mixture was then filtered through a fine muslin cloth and the filtrate collected and frozen (-20°C) until required for gas chromatography analysis. The residue was discarded. Duplicate water extracts were prepared for all the treatments. When required for analysis, the thawed samples were prepared, together with standards, as in Appendix B. From the tracings produced, the amounts of volatile fatty acids (VFA), lactic acid and ethanol were determined in g/kg fresh and dry weights.

2.5 Experiment 2: Dacron bag incubations.

A series of rumen incubation trials were carried out using the bag technique of Mehrez and Orskov (1977) to measure the degradability of the five major grain treatments (2 levels of Graintona, 2 levels of Sylade and Molasses). Three rumen fistulated, mature Suffolk wether sheep (average liveweight 65kg)

were used. They were housed in individual wooden pens, 1.2m wide, 2.6m long and 1.2m high, bedded with straw. The pens were open fronted and had holes in the side, enabling the animals to have visual contact with each other. The sheep were fed a maintenance ration of 1kg chopped hay and 400g of Dalgety - Shepherds Care 'Kelvin Ewe 16 Nuts' containing a teaspoonful of salt, split into two equal feeds per day; the first one at 08:45 and the second at 16:45.

The Dacron bags used in the incubation were 195mm long and 150mm wide with a pore size of 40 μ m. The rounded corners and double stitching of the bags facilitated easy removal of sample residues after incubation and avoided the loss of fine grain particles during incubation and subsequent washing. After the weight of an empty bag was recorded, approximately 6g fresh weight of a particular treated grain sample was added to the bag, any uncrimped grains being rejected. The weighing of bags and contents was performed accurately, to the nearest milligram. Bags which were to be incubated in the rumen were tied very securely and suspended with 30cm of nylon string. This length of string was sheathed with plastic tubing to avoid entanglement in the rumen and to allow free movement and easy mixing of the bags with rumen contents. Sets of five bags (one per treatment) were then grouped together and tied securely through a rubber bung which fitted exactly the rumen fistula of the sheep.

Each treatment in the trial was replicated in the three sheep for incubation times of: 4, 8, 16, 24 and 48 hours. On removal from the rumen after the appropriate incubation time, the bags were rinsed with running tap water to remove ruminal contents from the outside of the bag and then cold-water washed in a pre-set automatic washing machine (Zanussi Z915T, programme

B). They were then oven dried and the DM of the residues determined as in Section 2.4. Zero time bags (three per feed) were treated identically except that they were not incubated in the sheep.

The incubation trial which has been described, involving six time courses and the five major treatments, lasted for a period of seven days. The complete trial was then repeated two further times, so that at the end of the three, 7-day periods, each treatment had been incubated three times in three different sheep, that is, a total of nine times.

The residues from every incubated bag were milled through a 2mm screen using a 'Cyclotec 1092 Sample Mill' and collected in small (20cm³), capped plastic vials. Initially, it was hoped to keep the residues separate to allow differences between sheep to be analysed. Unfortunately, the amount of residue from a single bag was insufficient for the required analyses. This was particularly evident in the case of the 24 and 48 hour incubations where less than 0.5g DM of residue was retrieved. As a result of this, the residues of three replicated bags from the same period, incubation time and treatment were bulked and milled together. The residues were analysed for disappearances of OM and total nitrogen, and disappearances of PFOM were calculated for the five treatments over the six time courses as described in Section 2.4.

In-house computer programmes facilitated the calculation of the bag residue constituents. It was known that poor results would be obtained if more than five bags were incubated in the rumen of the sheep (Mehrez & Orskov, 1977). Therefore in this experiment, the Molasses was designated the control treatment.

Methods for interpretation and analysis of results.

The classical method of Orskov and McDonald (1979) was used to produce degradability curves for nitrogen, organic matter and protein-free organic matter. The original method involved relating the degradability of protein in feed samples, incubated in artificial fibre bags in the rumen, to the degradability of protein in the *in vivo* situation, by weighting such measurements according to rate of passage of digesta from the rumen to the lower reaches of the digestive tract. In this experiment, the same method is used to obtain, additionally, estimates of organic matter and protein-free organic matter degradability.

The percentage disappearance, p , of protein was obtained from the equation: $p = a + b (1 - e^{-ct})$, where a , b and c are constants fitted by an iterative least squares procedure, using the data obtained from the incubation trial at specific incubation times, t . p was measured under conditions preventing the passage of any nutrient particles from the rumen and is, therefore, an overestimate of the extent of degradation at any given time under normal conditions, when some particles would have already passed to the abomasum. In this investigation, the disappearance of nitrogen, organic matter and protein-free organic matter was obtained from the equation, which defines a fitted curve through the data obtained from the three sheep. Constant ' a ' refers to the percentage of total nutrient which disappears from the rumen very rapidly; within the period prior to the removal of the first bag from the rumen and is determined from the intercept of the curve on the Y - axis. Constant ' b ' refers to the percentage of total nutrient which disappears from the ruminal contents at a constant fractional rate ' c ' per unit time ' t '.

An analysis of variance was carried out on the disappearance figures obtained for the five different treatments at the various incubation times, using the EDEX 6H.2 statistical package. For nitrogen and protein-free organic matter, this analysis identified variance due to time and treatment, together with their main interactions. For organic matter, the analysis additionally identified the variance between sheep.

2.6 Experiment 3: The feeding trial.

Ten Blackface, Suffolk - cross wether sheep, aged nine months, which had previously been penned as a group were used. Each was weighed and placed in individual wooden pens (Experiment 2) in a small, well - ventilated sheep shed with an ambient temperature of 5°C. The average liveweight of the sheep was 34.5 kg. Each sheep had free access to water by means of an individual drinking bowl.

Whilst the sheep were settling into their pens, they were fed *ad libitum* hay and two 100g meals of moist 'Propcorn' barley grain (B.P. Nutrition, UK), which had a dry matter content of 766 g/kg, for three days. The two meals were then each increased to 200g to observe whether the sheep would successfully consume 400g of moist barley per day. As the 400g were easily consumed for a further two days, the feeding trial, involving the 5 major treatments contained in the large barrels and frozen untreated grain (Experiment 1), was initiated. On Day 162, one of the large barrels for each treatment was opened. 400g meals of each treatment were weighed out. Enough grain was removed to last seven days. The inner plastic liners of the barrels were vacuum resealed to aid preservation. A sack of control grain obtained from the freezer 4 days previously had been allowed to thaw. The contents of the sack were well mixed and control meals of 400g

were also weighed out to last seven days. The control feed was adequately thawed as there were no grain clumps or ice crystals visible. Any mouldy clumps which were found in the treated grain were discarded whilst measuring out the feed. The 400g meals were fed once per day at 08:45 hours and were immediately consumed. As there were no refusals for seven days and given the rapidity of feed intake, it was decided to increase meal size to 700g. This was fed once per day in order that any changes in rumen fermentation pattern and hence feed intake would be enhanced. Meals of 700g (fresh weight) were weighed out for ten days and the trial was restarted on Day 169. 15g of a mineral supplement per meal was also given to the sheep ('Sheep Standard', Scotmin, Maybole, Ayrshire), which provided the vitamins A, D₃ & E and the minerals Calcium, Phosphorus, Sodium Chloride (salt) Magnesium and Selenium.

All hay used in the trial was chopped and thoroughly mixed. This facilitated the ease of measuring and weighing. The diet was designed to provide an excess of forage so that refusals would occur. Therefore a forage to concentrate ratio of approximately 60:40 (FW) was chosen, so that for the 10 day *ad libitum* stages of the trial the daily hay allowance for each sheep was 1200g. During the restricted intake stages of the trial, this was reduced to 500g for each sheep, giving a forage to concentrate ratio of 40:60 (FW). It was not possible to use a basic Latin Square design for the trial as the number of individual pens to house the sheep was limited. Therefore, a balanced, incomplete block design was formulated involving 10 sheep, the six feeds and three 20 day periods. The design for the system of distribution of feeds is as follows in Table 2.3.

Table 2.3 Incomplete block design for crimped grain ensilage.

DESIGN FOR : 10 SHEEP
6 TREATMENTS
3 PERIODS

ALLOCATION:

GRAINTONA	100%	(GG)	(1)
GRAINTONA	50%	(G)	(2)
SYLADE	100%	(SS)	(3)
SYLADE	50%	(S)	(4)
MOLASSES		(M)	(5)
CONTROL		(C)	(6)

TREATMENTS				NUMERALS				
	1.	2.	3.		<u>PERIOD:</u>	1.	2.	3.
1.	SS	GG	G		1.	3	1	2
2.	S	SS	M		2.	4	3	5
3.	SS	S	C		3.	3	4	6
4.	GG	S	M		4.	1	4	5
5.	G	M	C	<u>SHEEP</u> <u>No.</u>	5.	2	5	6
6.	C	M	GG		6.	6	5	1
7.	GG	C	SS		7.	1	6	3
8.	S	G	GG		8.	4	2	1
9.	M	G	SS		9.	5	2	3
10.	G	C	S		10.	2	6	4

REPLICATION IS 80%

The balanced, incomplete block design is a recognised statistical technique, used in many agricultural applications when the blocks are not large enough to accommodate all the treatments. It was originally developed to analyse crop yields in fields of varying fertility but now finds applications in many areas of science and engineering. A full description of the applications of the incomplete block design, together with the methods for construction and formulation may be found by consulting the references Cox (1958); Fisher & Yates (1963) and John (1980).

Throughout the whole trial one concentrate meal was given at 08:45 hours to each sheep. In the first ten days of each experimental period, hay was given *ad libitum* and in the second ten days hay intake was restricted. Faecal collections were performed during the last three days of the restricted intake period. Regular dry matter measurements were performed on the hay

and grain to check for uniformity. Occasional pH measurements on the grain were also made to check for aerobic stability. Refusals were collected daily from each sheep and DM determinations made in order to find out their daily DM intake. During the three day collecting period, well mixed, representative samples of feed, faeces and refusals were taken for DM and subsequently OM determinations using the procedures described in Section 2.4.

Analyses of variance were carried out on the mean daily intake of the sheep. These analyses considered the 10 day *ad libitum* and restricted stages of each period, the former being considered in two 5 day halves (days 1-5 & 6-10) and the organic matter digestibility of the whole diet. It was hoped that variances which were due to sheep and to treatment would be identified. In the analyses, the daily hay DM intake was subsequently adjusted for the liveweight of the individual sheep in order to reduce the extent of between sheep variability.

PART THREE.

THE RESULTS.

3.1 Treatment Codes.

The complete set of treatments with their rates of application is repeated for reference, together with their individual **CODES**, which are used in all the subsequent figures and tables.

Treatment	Application rate (l / tonne)	CODE
-----	-----	-----
100% Graintona Plus	8.0	GG
50% Graintona Plus	8.0	G
100% Sylade 2	8.0	SS
50% Sylade 2	8.0	S
Cane Molasses	6.4	M
Cane Molasses	12.6	MM
Cane Molasses	6.4	
+ Forager Inoculant	2.5	MI
Cane Molasses	12.6	
+ Forager Inoculant	2.5	MMI
Forager Inoculant	2.5	I
Add - Safe	4.0	AS
Add - Safe	8.0	ASAS
Amylase	8.0	AM
Fresh, frozen	---	FF
Untreated	---	U

3.2 Experiment 1: Preservation studies.

All ensilations of the high moisture barley grain samples were completed successfully. On opening, all inner liners were intact and the ties had remained secure. Table 3.1 gives the chemical characteristics of the treated, ensiled grain at the end of the storage period.

Figure 3.1 shows the change in pH, measured in the small pH pots, during the ensilage process. The increased pH for the full strength treatments, GG and SS, corresponded to a high degree of moulding, as compared to the other treatments.

Overall, most of the barley treatments had an appetizing smell and it was thought that they would be an attractive and palatable feed for all groups of livestock. It was noted how the ensiled grain, on opening, had retained the smell of the original

Figure 3.1 Variation of pH in samples of crimped, ensiled grain with time.

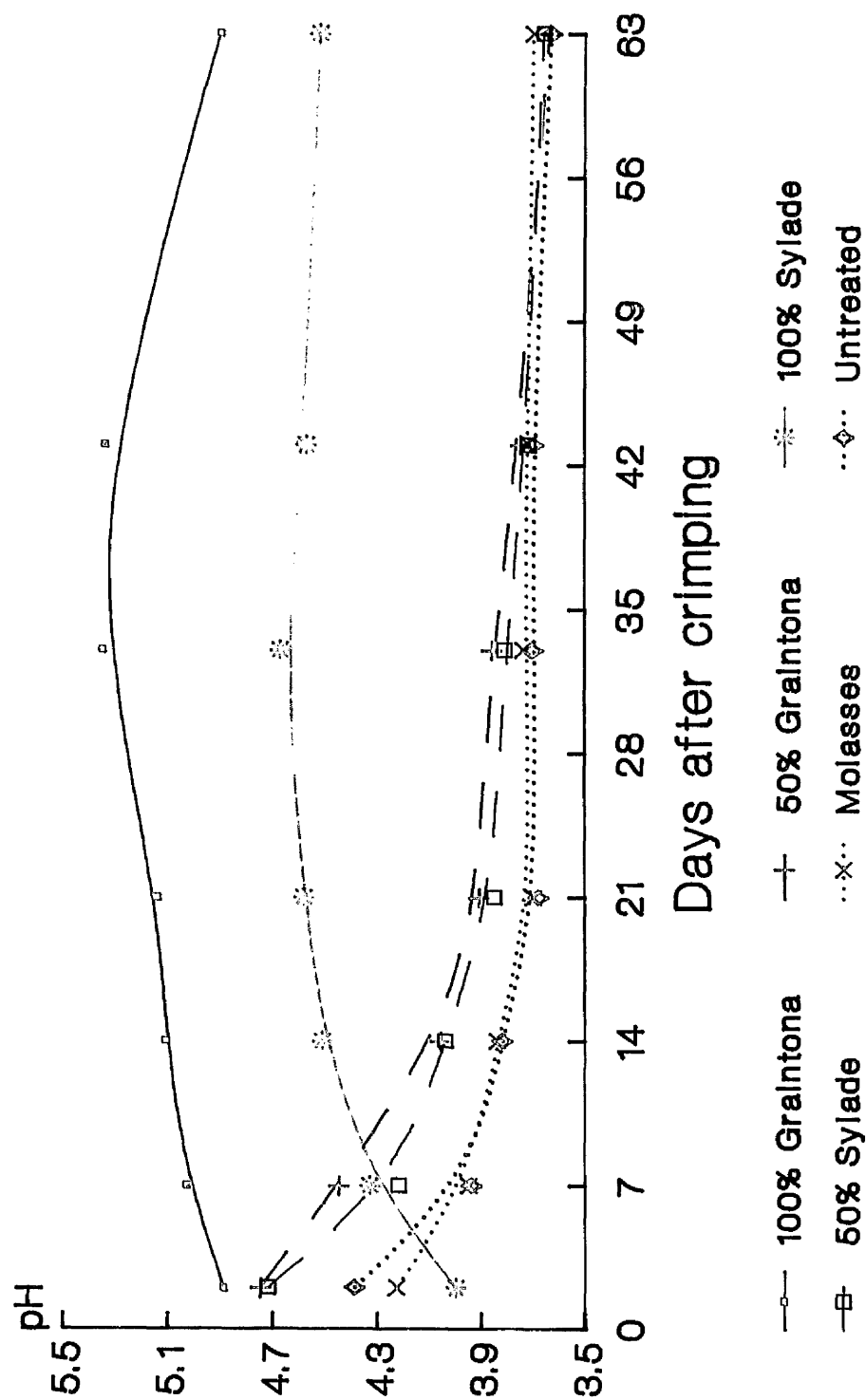


Table 3.1 Composition of both major and minor treatments.

Treatment	DM	pH	Waste	OM	Nitrogen	PFOM
	(g/kg)		(%)	(g/kgDM)	(g/kgDM)	(g/kgDM)
-----	-----	-----	-----	-----	-----	-----
Graintona 100%	561	5.37	1.3	972	20.1	847
Graintona 50%	548	3.76	0.2	972	19.0	853
Sylade 2 100%	550	4.58	4.9	972	20.2	846
Sylade 2 50%	543	3.80	*	970	20.7	841
Molasses	542	3.71	*	969	18.5	853
Untreated	541	3.68	2.0	971	20.0	846
Twice molasses	524	3.79	0.5	969	18.3	854
Add - Safe	540	3.91	*	970	18.7	853
Twice Add-Safe	553	3.82	1.2	971	19.2	851
Molasses						
+Innoculant	525	3.75	0.9	968	18.8	850
Twice molasses						
+Innoculant	528	3.67	1.0	967	18.9	849
Innoculant	530	3.80	0.9	969	19.0	850
Amylase	525	3.77	0.9	967	19.6	844
Fresh frozen	565	4.67	---	972	17.1	865

* Indicates less than 0.1% wastage.

treatments, although to a lesser extent, indicating that the ensilation had been airtight. The Graintona and Sylade 100% treatments showed an amount of moulding and in these areas the grain had a smell which was doughy or similar to mouldy cheese. Both the moulding and the associated odour became increasingly common when these treatments were weighed out and stored for a number of days (even in airtight containers) for the feeding trial (Experiment 3). The untreated ensiled grain smelt very doughy, similar to mouldy bread. The molasses treatments, including those containing the innoculant, had an extremely appetizing smell, which was rather fruity and alcoholic. The grain also had a much higher moisture content than the other treatments, as shown by the low dry matter content of those samples. The Add-Safe treatment, especially at the high application rate, smelt highly acidic.

Figures 3.2.1 to 3.2.6 show the results of the gas chromatography analysis of the water extracts which were obtained from the treated barley grain samples. The overall total fermentation acid content is shown in Figure 3.2.1. Isobutyric acid is shown highlighted for the Graintona treatments, as for the high application rate it comprised more than half the total fermentation acid content (Appendix D). Figures 3.2.2 and 3.2.3 highlight the ratio of lactic to total acid and the lactic acid content of the high moisture barley samples respectively. The suppression of lactic acid fermentation by the two full strength additives, GG and SS, is immediately apparent in Figure 3.2.3. Figure 3.2.4 shows the acetic acid content of all the barley grain treatments. Again, SS and GG restricted fermentation most effectively. Figure 3.2.5 shows the extent of alcohol production in relation to the total fermentation acids. Figure 3.2.6 highlights the actual ethanol content of the individual grain samples. It can be seen that all the treatments containing molasses had high ethanol contents.

Figures 3.3.1 and 3.3.2 give the results of the aerobic stability study in graphical form, for both white and blue mould development within the glass tubes. Although the development of mould with time is a continuous variable, when plotting the graph it was found that the fit of the curves to the data points was unsatisfactory. The graphs were therefore plotted with lines to give a more representative figure and this method of display additionally shows the variability of mould growth with time.

The buffering capacity of untreated barley was determined by autotitration. This barley grain macerate was found to require 175 milli - equivalents of 1 molar sodium hydroxide per kg dry matter to change the pH of the solution from 4.0 to 6.0.

Figure 3.2.1 Grain total fermentation acids.

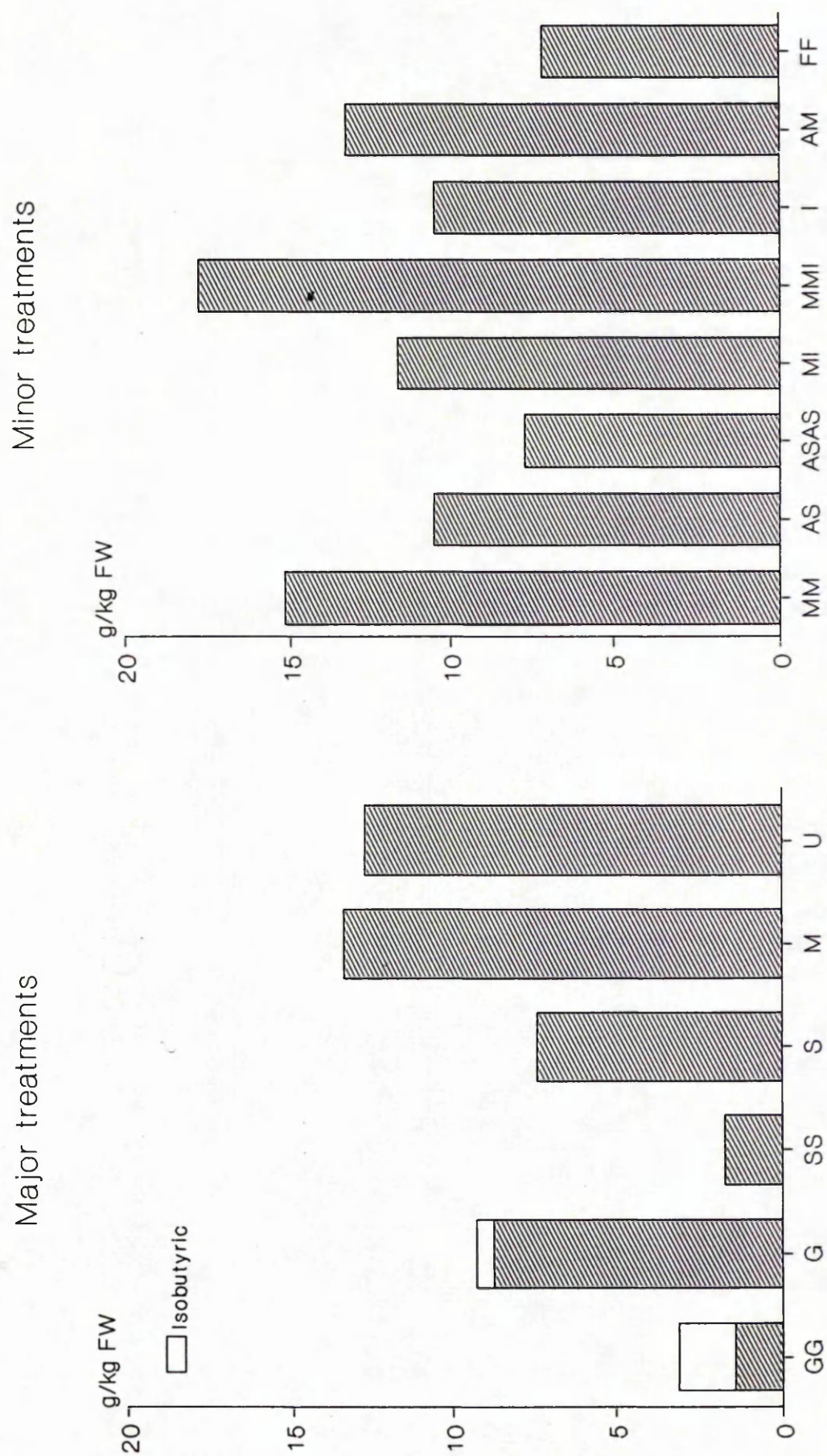


Figure 3.2.2 Lactic acid to total fermentation acid ratio.

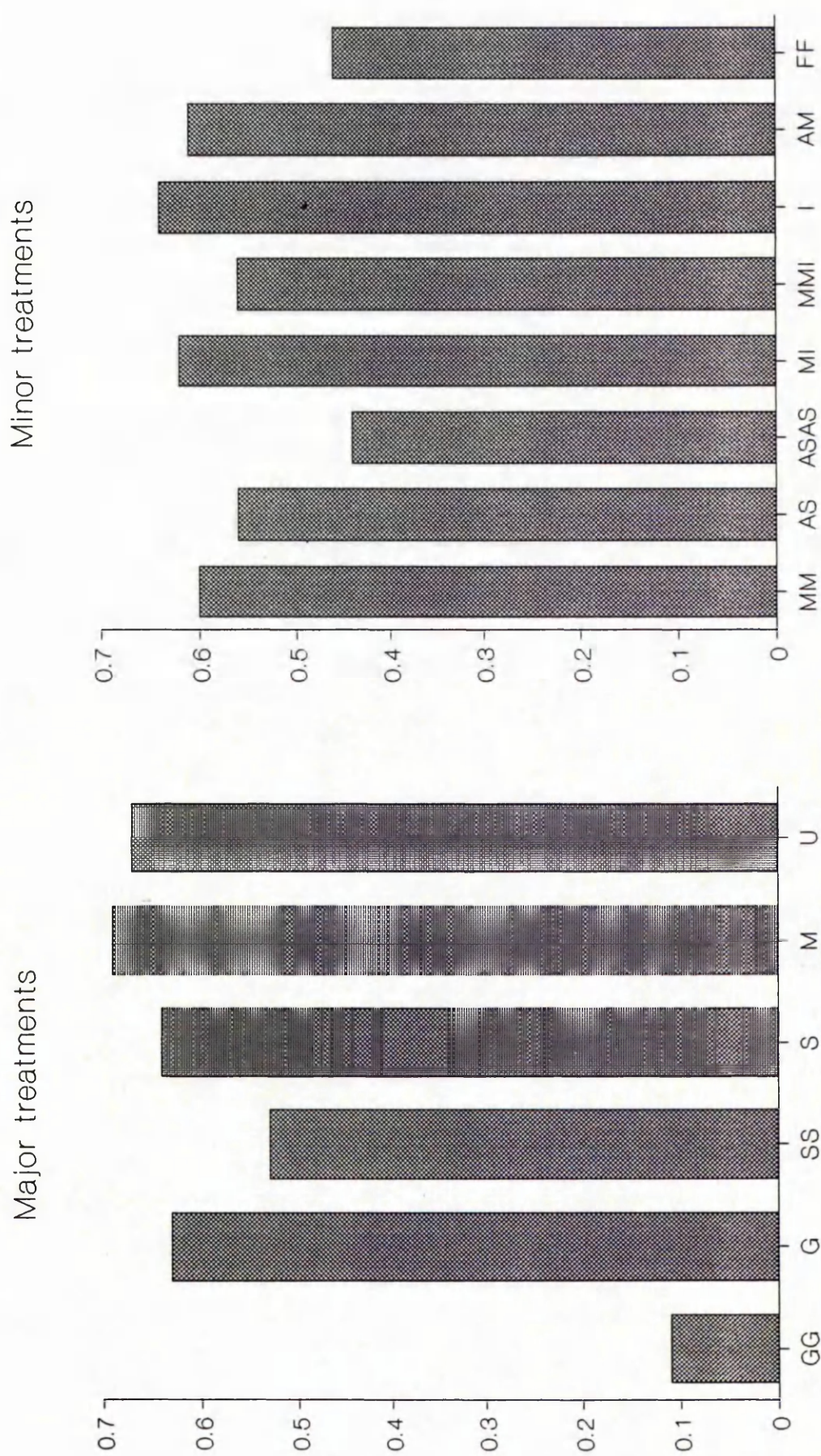


Figure 3.2.3 Lactic acid content.

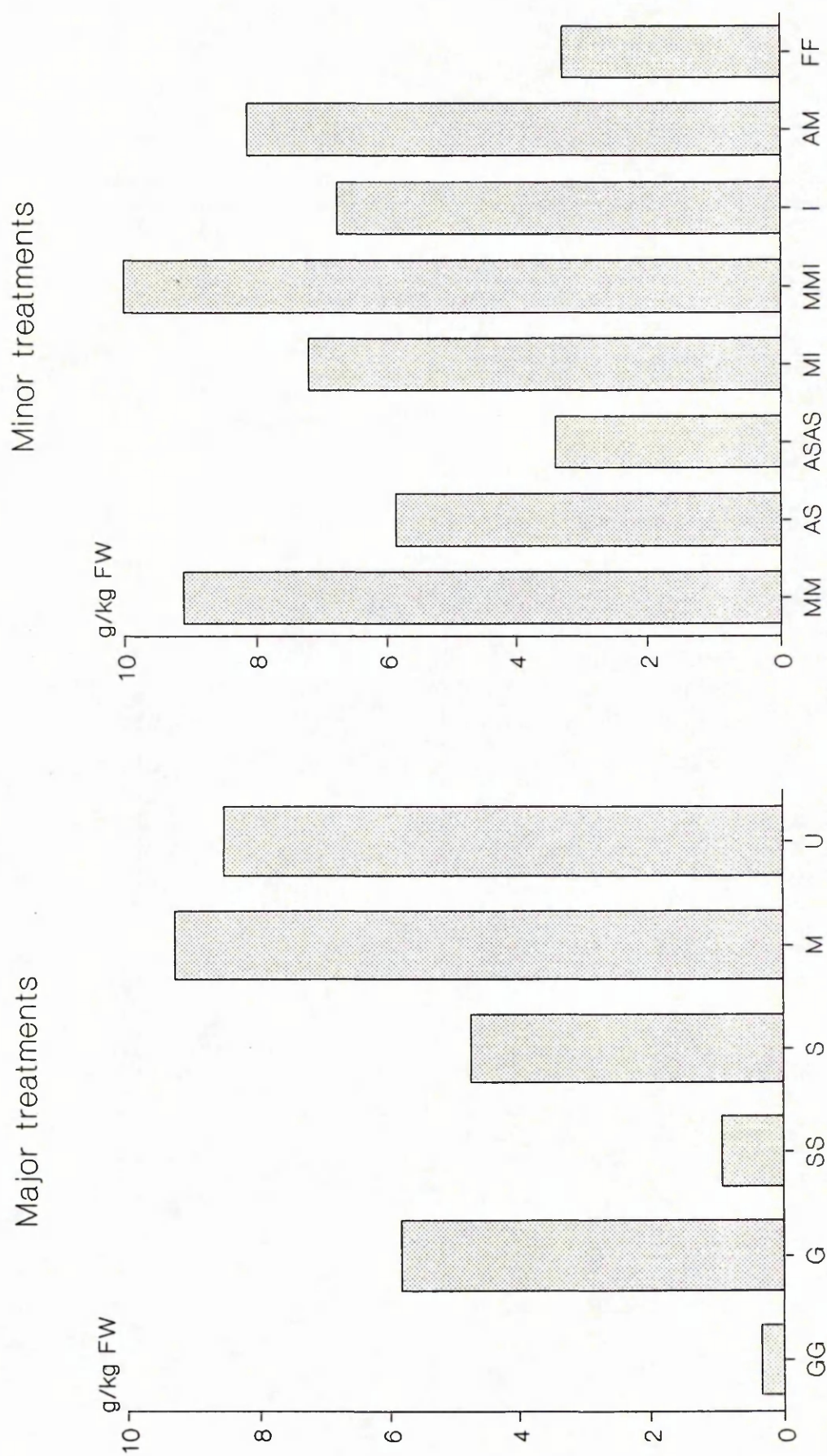


Figure 3.2.4 Acetic acid content.

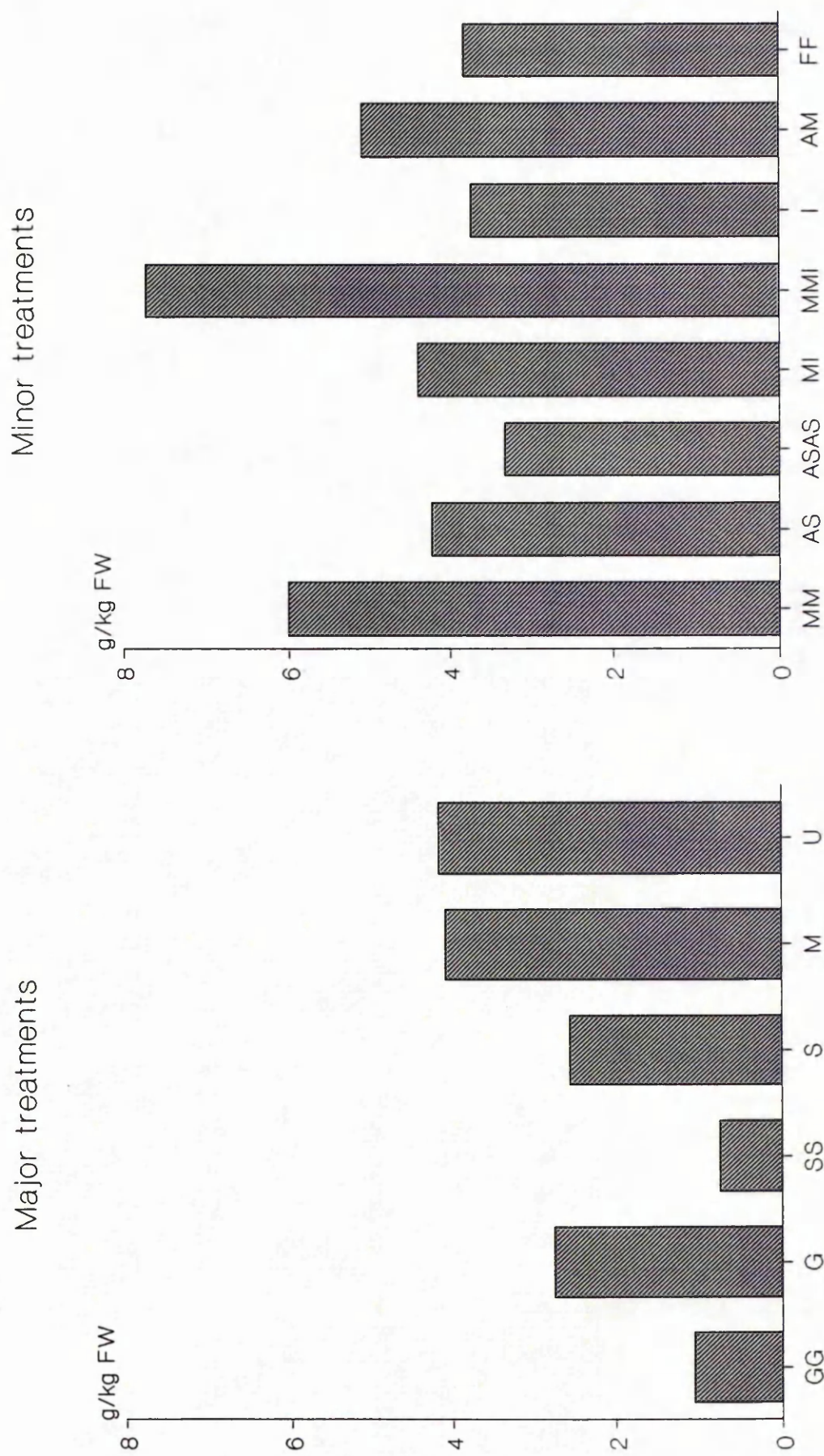


Figure 3.2.5 Total fermentation acid to alcohol ratio.

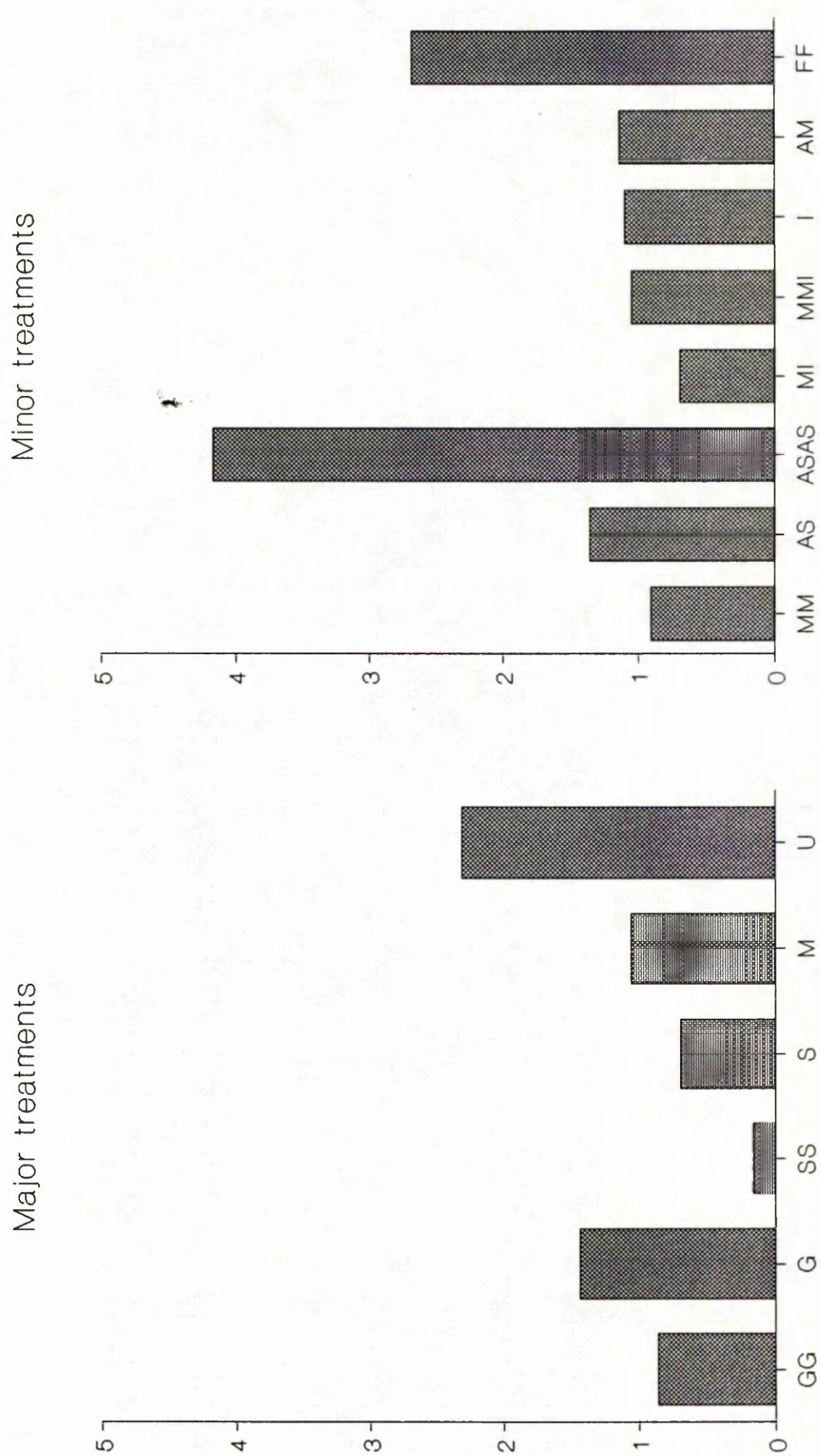
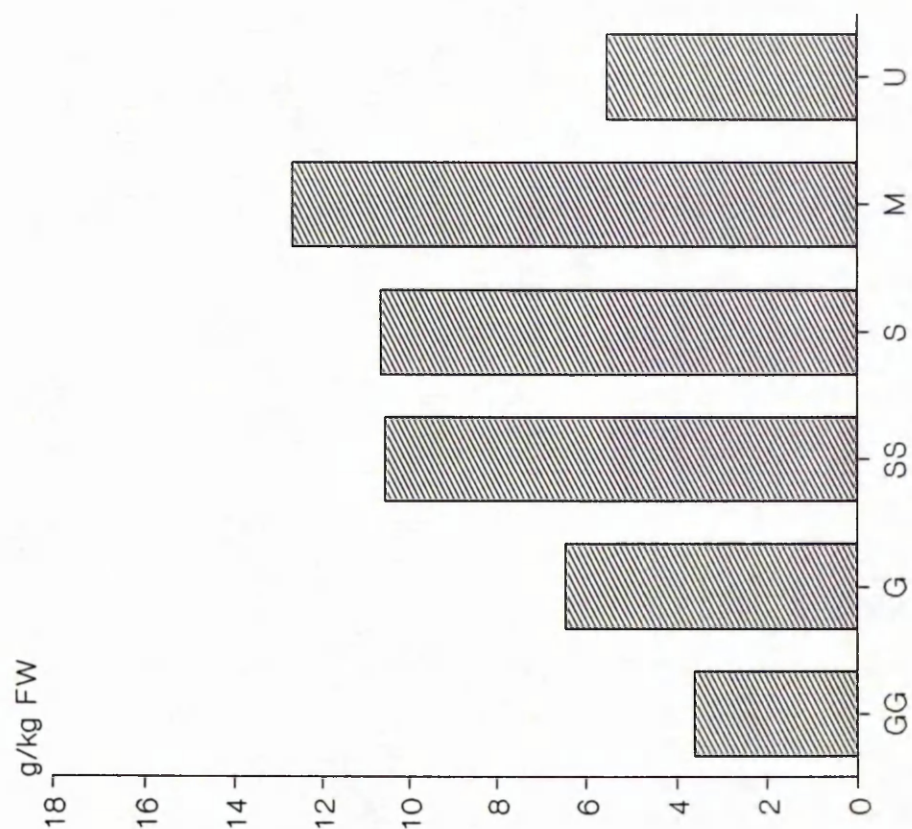


Figure 3.2.6 Ethanol content.

Major treatments



Minor treatments

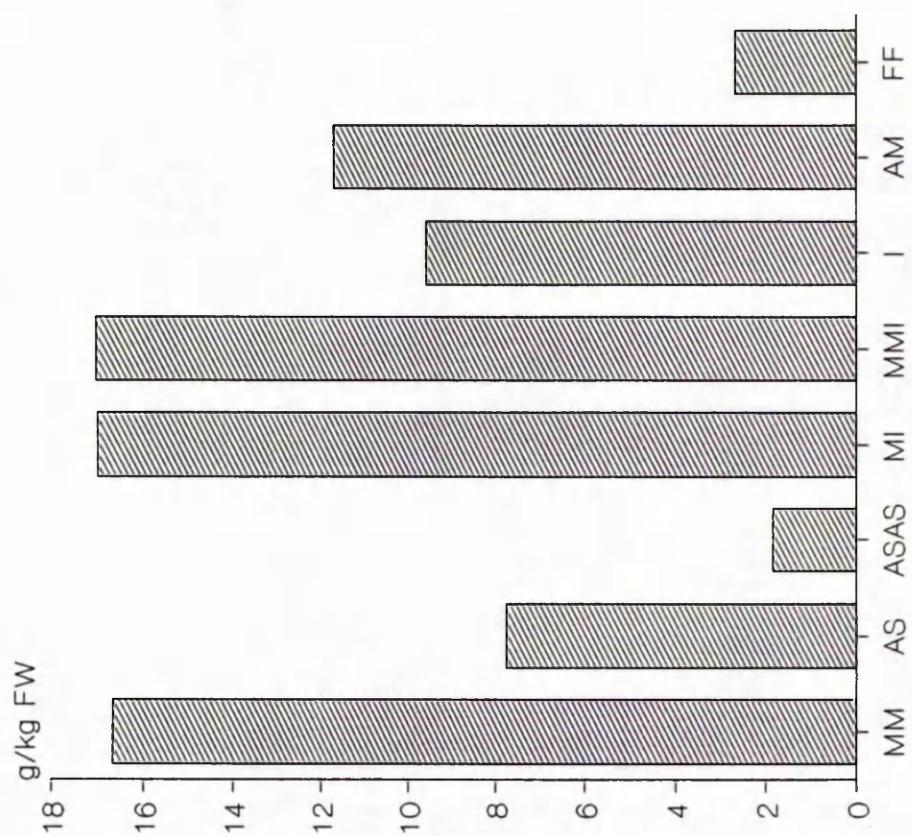


Figure 3.3.1 Treatment aerobic stability
White mould

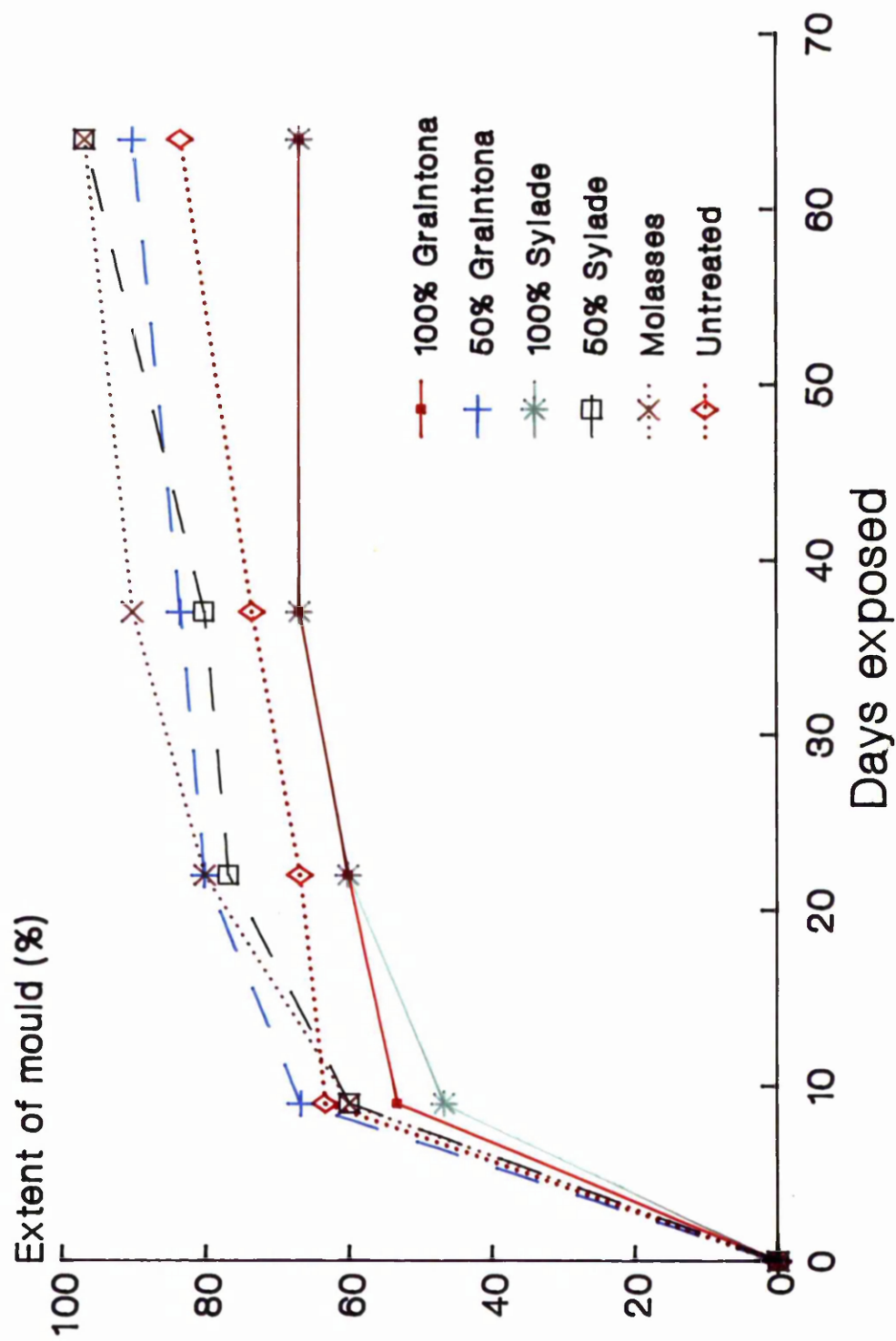
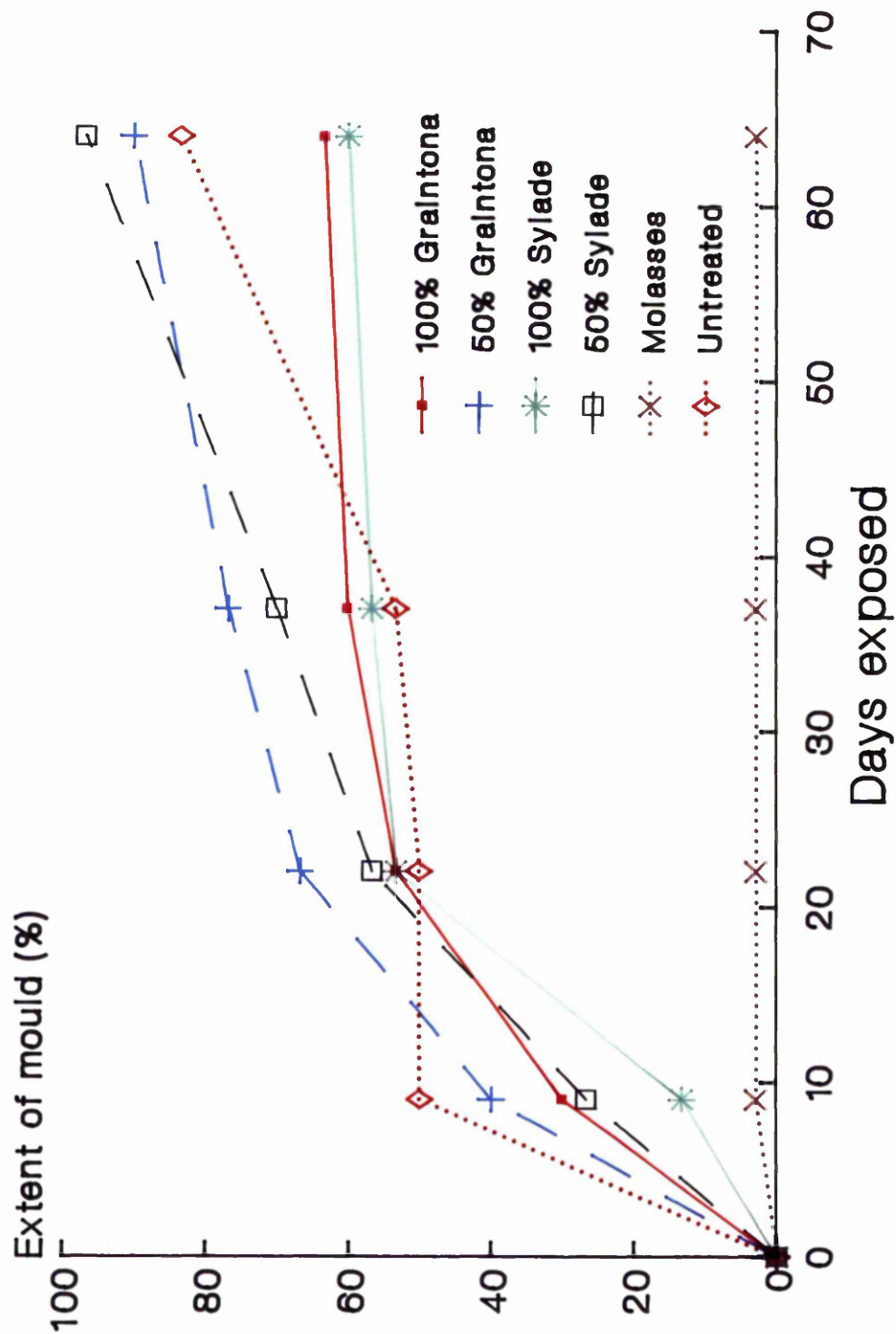


Figure 3.3.2 Treatment aerobic stability
Blue mould



3.3 Experiment 2: Dacron bag incubations.

All *in sacco* rumen incubations were carried out successfully in the three Suffolk wethers. The sheep remained healthy during the incubation trial and there was no loss of appetite or feed refusals. Tables 3.2 and 3.4 give the analyses of variance for the mean of the three sheep for nitrogen and protein-free organic matter digestion respectively. Table 3.3 gives the analysis of variance for organic matter digestion using the individual data from the sheep. The individual treatment and time results are also shown with significant differences marked by superscripts. In all cases it can be seen that the greatest differences between treatments occurred at short rumen incubation times and the effects of treatment became less significant after 24 hours of rumen incubation. The percentage mean effective degradability for each parameter was calculated from the fitted constants **a**, **b** & **c** (Orskov and McDonald, 1979). These constants are shown, together with the percentage mean effective degradability for two rumen outflow rates: 4% per hour and 8% per hour.

Figures 3.4 to 3.6 show the data from Tables 3.2 to 3.4 in the form of fitted curves for nitrogen, organic matter and protein-free organic matter respectively. The solid lines represent the full strength (100%) treatments and the dotted lines the half strength (50%) treatments. The way in which these lines pair according to strength of treatment is clearly obvious in all three figures. The fine dotted line represents the molasses treatment, which served as the control for this investigation. This treatment, after 10 hours, showed the greatest rumen degradation of all the grain samples.

Table 3.2 In sacco nitrogen disappearance (%).Analysis of variance (mean of three sheep).

Source of variation	DF	SS	MS	F
Period	2	63.93	31.96	1.35
Treatment	4	2529.56	632.39	26.77 ***
Error (1)	8	189.02	23.63	
Time	5	5436.14	1087.23	230.38 ***
Time/Treatment	20	751.08	37.55	7.96 ***
Error (2)	50	235.96	4.72	
Total	89	9205.70		

*** Significant at $P < 0.001$.Individual treatments and times.

Time (hr)	GG	G	SS	S	M
0	54.30 ^a	72.90 ^c	64.20 ^b	75.90 ^{cd}	79.20 ^d
4	66.43 ^a	83.53 ^b	69.07 ^a	81.53 ^b	84.40 ^b
8	69.47 ^a	85.23 ^{cd}	75.97 ^b	83.57 ^c	88.33 ^d
16	77.63 ^a	87.97 ^b	81.97 ^a	88.00 ^b	90.27 ^b
24	86.40 ^{ab}	91.57 ^c	85.03 ^a	90.93 ^{bc}	91.77 ^c
48	90.70 ^a	93.00 ^a	91.87 ^a	92.53 ^a	95.13 ^a

Least significant difference = 4.60.

Means not followed by the same superscript in the same row differ significantly, $P < 0.05$.Values for the constants a, b & c in the equation $p=a+b(1-e^{-ct})$.

Constant	GG	G	SS	S	M
a	55.6	74.0	64.7	76.9	79.7
b	37.7	17.9	29.6	16.5	13.4
c	0.060	0.158	0.053	0.071	0.114

Mean effective degradability (%) measured in sacco.

Outflow rate (% / hour).	GG	G	SS	S	M
4	78	88	82	87	90
8	72	86	76	85	88

Table 3.3 In sacco organic matter disappearance (%).Analysis of variance (figures for individual sheep).

Source of variation	DF	SS	MS	F	
Sheep	2	157.16	78.58	12.10	***
Period	2	88.02	44.01	6.77	**
Treatment	4	3583.03	895.76	137.85	***
Sheep/Treatment	8	59.56	7.45	1.15	
Error (1)	16	103.97	6.50		
Time	5	22426.57	4485.31	543.80	***
Time/Treatment	20	865.22	43.26	5.25	***
Error (2)	212	1748.60	8.25		
Total	269	29032.15			

*** Significant at $P < 0.001$.** Significant at $P < 0.01$.Individual treatments and times.

Time (hr)	GG	G	SS	S	M
0	50.10 ^a	64.93 ^c	52.00 ^a	57.20 ^b	64.73 ^c
4	62.14 ^a	73.93 ^c	62.40 ^a	68.39 ^b	72.34 ^c
8	67.34 ^a	75.88 ^c	67.14 ^a	72.42 ^b	76.91 ^c
16	72.03 ^a	78.67 ^{bc}	73.88 ^a	76.44 ^b	81.23 ^c
24	79.37 ^b	83.47 ^c	76.32 ^a	79.61 ^b	82.88 ^c
48	84.80 ^a	86.64 ^{ab}	85.26 ^a	85.66 ^a	88.77 ^b

Least significant difference = 2.63.

Means not followed by the same superscript in the same row differ significantly, $P < 0.05$.Values for the constants a, b & c in the equation $p=a+b(1-e^{-ct})$.

Constant	GG	G	SS	S	M
a	50.7	65.5	53.7	58.8	64.5
b	34.0	20.7	32.6	25.6	22.8
c	0.077	0.085	0.057	0.088	0.090

Mean effective degradability (%) measured in sacco.

Outflow rate (% / hour).	GG	G	SS	S	M
4	73	80	73	76	80
8	67	76	67	72	77

Table 3.4 In sacco protein-free organic matter disappearance (%).Analysis of variance (mean of three sheep).

Source of variation	DF	SS	MS	F
Period	2	53.46	26.73	4.99 *
Treatment	4	971.38	242.85	45.36 ***
Error (1)	8	42.83	5.35	
Time	5	7144.20	1428.84	478.11 ***
Time/Treatment	20	284.80	14.24	4.77 ***
Error (2)	50	149.43	2.99	
Total	89	8646.11		

* Significant at $P < 0.05$.*** Significant at $P < 0.001$.Individual treatments and times.

Time (hr)	GG	G	SS	S	M
0	49.40 ^a	63.70 ^c	57.20 ^b	54.50 ^b	62.80 ^c
4	61.57 ^a	72.67 ^c	61.47 ^a	66.40 ^b	70.77 ^c
8	67.03 ^a	74.63 ^c	65.77 ^a	70.70 ^b	75.33 ^c
16	71.20 ^a	77.47 ^{cd}	72.57 ^{ab}	74.70 ^{bc}	80.00 ^d
24	78.40 ^b	82.30 ^c	75.00 ^a	77.80 ^{ab}	81.67 ^c
48	83.97 ^a	85.70 ^{ab}	84.33 ^a	84.63 ^a	87.83 ^b

Least significant difference = 3.02.

Means with different superscripts in the same row differ significantly, $P < 0.05$.Values for the constants a, b & c in the equation $p = a + b(1 - e^{-ct})$.

Constant	GG	G	SS	S	M
a	51.4	65.2	57.6	56.0	63.8
b	33.1	20.9	34.6	27.9	23.7
c	0.069	0.071	0.031	0.080	0.075

Mean effective degradability (%) measured in sacco.

Outflow rate (% / hour).	GG	G	SS	S	M
4	72	79	73	75	79
8	67	75	67	70	75

Figure 3.4 Nitrogen digestion,
in sacco

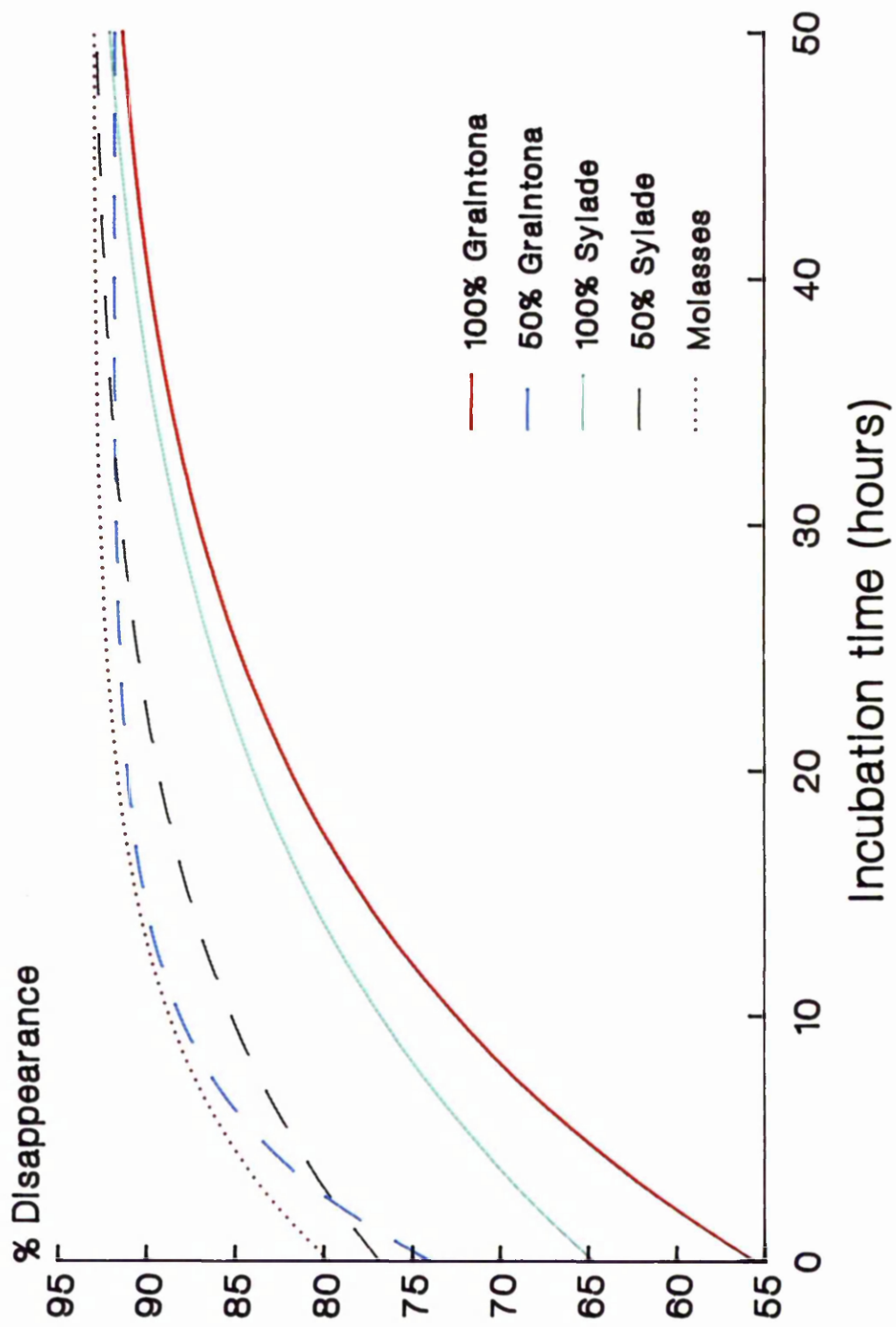


Figure 3.5 Organic matter digestion,
In sacco

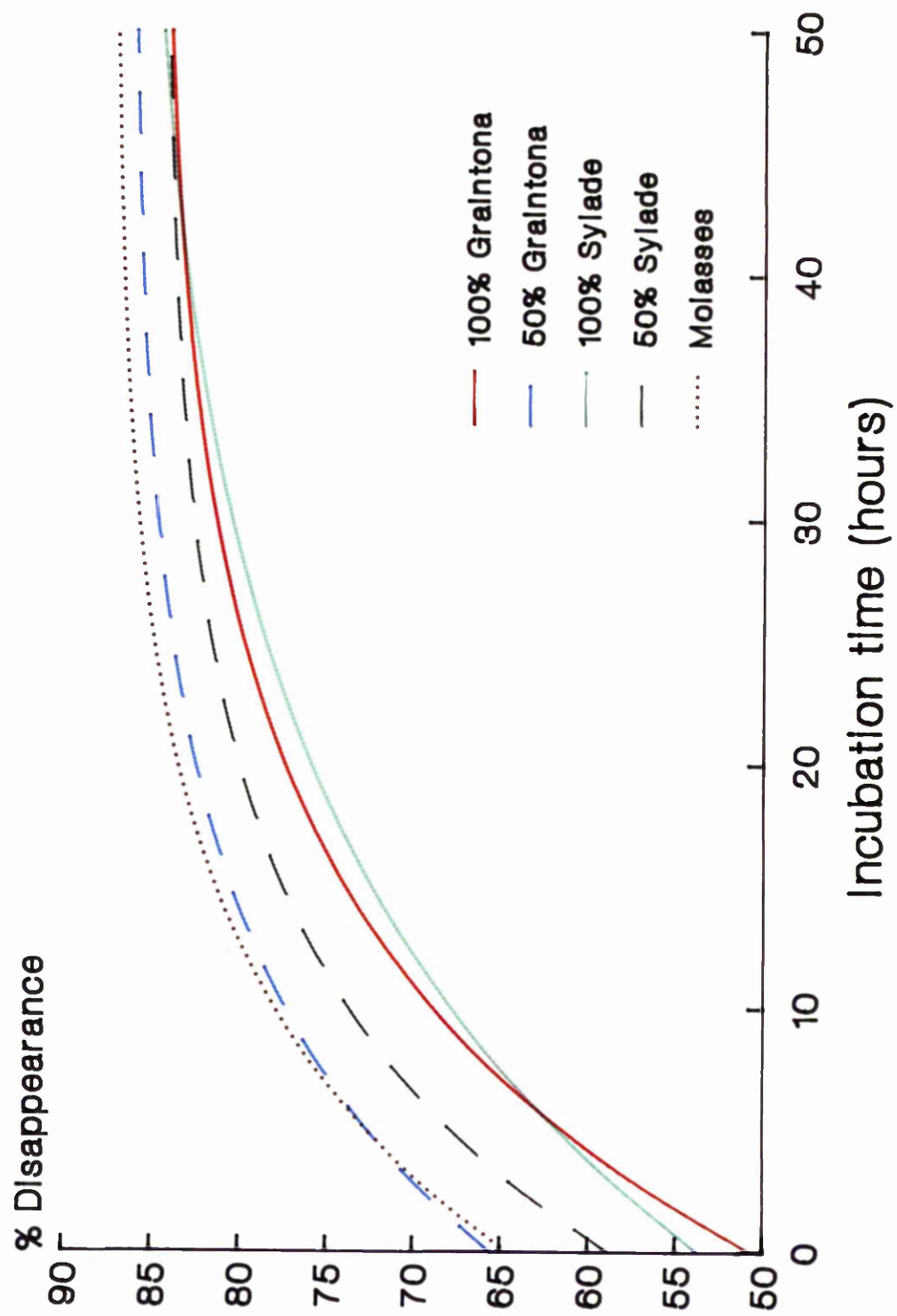
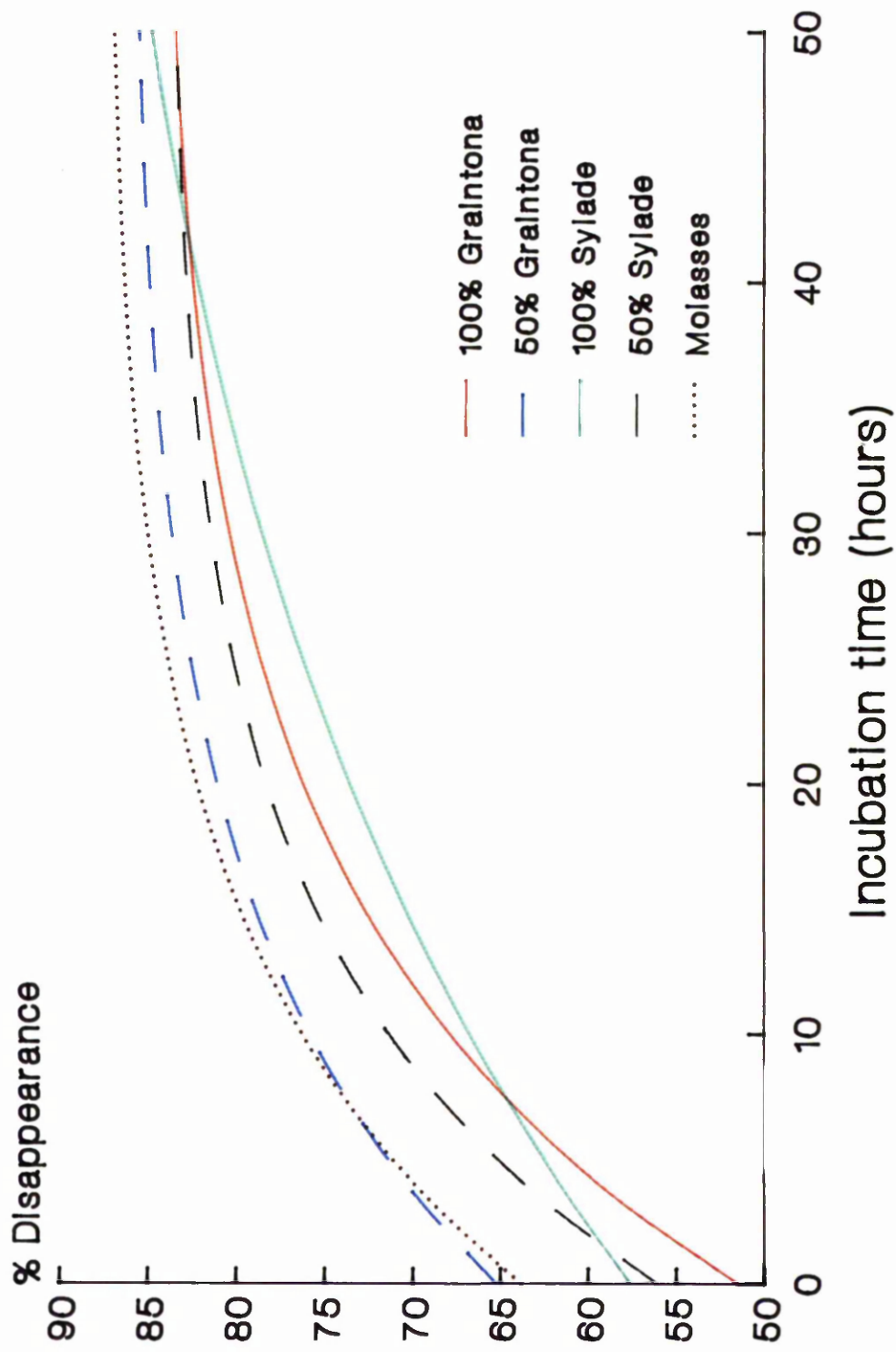


Figure 3.6 *In sacco* digestion of protein - free organic matter.



Experiment 3: The feeding trial.

The trial involving the ten individually penned yearling sheep was completed according to plan. Only one concentrate refusal occurred (in Period 2) and this was immediately after Sheep 9 had been sheared. The poor condition of the fleece made shearing a necessity. Apart from this refusal, no further noticeable intake effects were observed for Sheep 9 throughout the remainder of the feeding trial. The average liveweight of the sheep at the end of the trial was 41.0kg. Table 3.5.1 shows the analysis of variance for sheep hay dry matter intake (HDMI) for days 1 - 5 over the three 20 day periods. Although a significant effect ($P < 0.05$) was found for sheep, an effect due to treatment was not observed during the initial five days of a new period. The individual intake data for the sheep over the first five days of the three periods is also shown. This individual data is illustrated because in the incomplete block design, some treatments were not tested by each sheep in a particular period. The treatments not tested are represented by an asterisk (*). The mean hay dry matter intake is given, together with a corrected mean, generated by GENSTAT analysis, which would apply if each individual sheep had tested each treatment. The corrected mean is used in any analyses or comparisons.

Table 3.5.2 gives the analysis of variance and HDMI for the sheep for days 6 - 10 over the three periods. An increase in hay intake for each treatment can be seen compared with days 1-5, together with a larger coefficient of variation.

Table 3.5.3 gives the analysis of variance and HDMI for the ten day restricted hay intake session. Although the figures are similar and hay intakes are small, the analysis and individual figures are included for completeness.

Table 3.5.1 Sheep hay DM intake (g/kg LW). Days 1-5.

Analysis of variance.

Source of variation	DF	SS	MS	F
Sheep	9	109.71	12.19	3.49 *
Treatment	5	36.86	7.37	2.11
Error	15	52.36	3.49	
Total	29	198.93		

Coefficient of variation = 10.90%

* Significant at $P < 0.05$.

Individual sheep and treatment figures.

Sheep	GG	G	SS	S	M	U
1	18.62	22.23	20.99	*	*	*
2	*	*	14.74	15.44	16.79	*
3	*	*	17.68	12.68	*	19.11
4	16.09	*	*	13.25	15.78	*
5	*	17.48	*	*	19.22	16.70
6	11.34	*	*	*	16.08	15.61
7	16.19	*	20.00	*	*	17.63
8	17.57	15.38	*	18.06	*	*
9	*	18.38	21.51	*	20.13	*
10	*	13.36	*	16.81	*	19.56
Mean :	15.96	17.37	18.98	15.25	17.60	17.72

Corrected

mean:	15.86	15.86	18.20	16.02	18.44	18.51
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SED = 1.32

Table 3.5.2 Sheep hay DM intake (g/kg LW). Days 6-10.

Analysis of variance.

Source of variation	DF	SS	MS	F
Sheep	9	91.84	10.20	2.15
Treatment	5	5.95	1.19	0.25
Error	15	71.20	4.75	
Total	29	168.99		

Coefficient of variation = 11.77%

Individual sheep and treatment figures.

Sheep	GG	G	SS	S	M	U
1	20.68	23.48	22.00	*	*	*
2	*	*	17.12	15.62	18.29	*
3	*	*	17.45	18.03	*	21.61
4	16.24	*	*	12.93	18.42	*
5	*	17.59	*	*	15.30	18.12
6	18.68	*	*	*	16.50	16.10
7	17.04	*	21.80	*	*	18.38
8	20.74	18.04	*	17.32	*	*
9	*	20.08	21.93	*	19.35	*
10	*	16.91	*	21.86	*	17.92
Mean:	18.68	19.22	20.06	17.15	17.57	18.42

Corrected

mean:	18.67	18.27	19.19	17.58	18.63	18.77
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SED = 1.54

Table 3.5.3 Sheep hay DM intake (g/kg LW). Restricted session.

Analysis of variance.

Source of variation	DF	SS	MS	F
Sheep	9	14.67	1.63	3.75 *
Treatment	5	1.73	0.35	0.80
Error	15	6.52	0.43	
Total	29	22.91		

Coefficient of variation = 6.91%

* Significant at $P < 0.05$.

Individual sheep and treatment figures.

Sheep	GG	G	SS	S	M	U
1	10.44	9.30	10.13	*	*	*
2	*	*	10.68	9.65	11.12	*
3	*	*	9.34	9.95	*	10.18
4	8.59	*	*	8.19	8.65	*
5	*	8.03	*	*	7.68	10.06
6	8.64	*	*	*	9.43	9.43
7	9.56	*	9.87	*	*	9.05
8	10.02	10.25	*	9.18	*	*
9	*	10.52	10.81	*	10.55	*
10	*	8.19	*	9.71	*	9.04
Mean:	9.45	9.26	10.17	9.33	9.49	9.55

Corrected

mean:	9.63	9.12	9.65	9.31	9.56	9.97
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SED = 0.466

Table 3.6 Whole diet organic matter digestibility (%).

Analysis of variance.

Source of variation	DF	SS	MS	F
Sheep	9	49.86	5.54	0.41
Treatment	5	81.21	16.24	1.21
Error	15	202.00	13.47	
Total	29	333.07		

Coefficient of variation = 4.93%

Individual sheep and treatment figures.

Sheep	GG	G	SS	S	M	U
1	71.60	70.00	76.20	*	*	*
2	*	*	76.50	75.20	72.60	*
3	*	*	75.20	75.60	*	76.40
4	76.80	*	*	76.70	75.60	*
5	*	77.90	*	*	66.90	79.10
6	76.30	*	*	*	77.60	73.80
7	71.00	*	77.70	*	*	75.70
8	76.50	77.90	*	65.20	*	*
9	*	72.40	75.10	*	70.60	*
10	*	69.80	*	74.00	*	76.70
Mean:	74.44	73.60	76.14	73.34	72.66	76.34
Corrected						
mean:	74.25	74.76	76.94	72.70	71.65	76.20

SED = 2.60

Appendix E contains the tables which show the individual daily intakes for each individual sheep during days 6 - 10 of each period. These days were chosen because they were thought to represent the ones most likely to illustrate the differences between treatments, as the sheep had been accustomed to their new diet for five days.

The results for the organic matter digestibility of the whole diet are shown in Table 3.6. The corrected means have been calculated in the same way as the previous ones to account for the missing sheep data.

PART FOUR.

DISCUSSION.

4.1 Experiment 1: Preservation studies.

Monitoring the pH conditions throughout the ensiling period, using the small pH pots, showed a reduction in pH for all except the two full strength treatments. These remained high and continued to rise (Figure 3.1), until a plateau was reached, which then declined. The tables in Appendix C show that these full strength treatments were associated with a significant amount of moulding and pH difference. Also, the individual pot silos showed significantly similar pH readings. Given the rather arbitrary method of measuring the amount of moulding, the similarity between the amount of mould in each of the three pot silos of the same treatment is also worthy of note. Table 3.1 indicates the highest dry matter content of the treated samples was provided by the Grintona 100%. From these results, the higher strength additions gave the greatest dry matter retention, but with a higher associated risk of mouldy waste. From the onset of the initial research, it was immediately apparent that there was a great similarity between the two 100% Grintona and Sylade treatments, which continued throughout the experimental analyses.

The gas chromatography analyses proved to be worth the detailed preparation, as the results indicated interesting differences between treatments. The overview of total grain water extract fermentation acids (Figure 3.2.1), distinctly shows how the 100% Grintona and Sylade treatments (GG & SS) suppressed production of fermentation acids and also acted as effective fermentation inhibitors, which should indicate a more stable product (McDonald, 1981). When the treatments were used at half the recommended strength, 50%, the Sylade appeared to be the more inhibitory, allowing less total fermentation acids to be formed than the Grintona. For the Grintona treatments, isobutyric acid

is highlighted, as it was believed that this was a component of the original additive. Isobutyric acid contents were negligible in all other treatments (Appendix D). Assuming this to be the case, it is apparent that the commercial additive, GG, was the most successful in reducing total fermentation acids in the barley grain. It is, however, interesting to note that Barry and Fennessy (1972) indicated that isobutyric acid is evidence of an unsatisfactory secondary fermentation in grass silage and that this was found to be greater in formaldehyde-treated grass silage than in untreated grass silage. Given the appearance of mould and the fact that Grintona is based on formaldehyde, this would merit further investigation. The Molasses treatment (M), stimulated fermentation, as expected from a carbohydrate source, giving more total fermentation acids than the untreated barley grain sample. Recalling that the five major treatments were applied at the farm and that the minor treatments were applied two days later (Section 2.3), it could be postulated that the amount of acid in the fresh, frozen grain should be taken as the control for the minor treatments and the untreated barley grain samples. The acids generated in the fresh, frozen grain (7.1 g/kg FW) would have been formed during the transportation and while the grain was standing for 24 hours, before it could be frozen. However, there are too many conflicting factors to justify this. The minor treatments were applied over a number of days to thawed batches of grain, introducing a different time scale and different environmental conditions. The effect of freezing on the untreated grain is a variable which cannot be quantified in this experiment. As it was not possible to complete the ensilations simultaneously, freezing was necessary to prevent grain deterioration. Figure 3.2.1 clearly shows the greater amount of

total fermentation acids formed from all the minor molasses treatments (MM, MI, MMI). Add-Safe at the high level (8l/t: twice the manufacturer's recommended level), has acted as an effective fermentation inhibitor, allowing production of total fermentation acids only 0.5 g/kg FW above that of the fresh, frozen. The lower level of Add-Safe (AS), gave an identical result to the Forager bacterial inoculant.

As it is well known that lactic acid bacteria are the most desirable in an ensiled crop (McDonald, 1981), the lactic to total fermentation acid ratio should ideally be high, especially for the fermentation stimulants. Figure 3.2.2 distinctly shows that this has been achieved for all treatments except 100% Graintona. This has acted as a much stronger fermentation inhibitor than 100% Sylade, which has a ratio five times that of 100% Graintona. All the major treatments, apart from GG, contained more than 50% of total fermentation acids as lactic acid. The same statement is true for the minor treatments, except for high Add-Safe, which actually contained slightly less lactic acid (44%) than the frozen sample (46%). The 6.4 l/t molasses application rate had a greater lactic acid content than the high level molasses. This indicates that the high rate may not stimulate lactic acid bacteria further, as other factors may have limited the fermentation. Figure 3.2.3 shows the lactic acid content of the individual treatments, which emphasizes the previous point. Graintona, applied at the manufacturer's recommended rate, is the most effective in inhibiting lactobacilli, three times more so than Sylade at the same rate. Clearly, 50% Graintona and Sylade do not act nearly so effectively as the 100% treatments, in restricting lactic acid fermentation. Also worthy of note is the high lactic acid (and

high ratio of lactic to other acids), given by the untreated barley grain sample, indicating that lactobacilli are dominant in the natural state. In order to increase lactic acid production above that observed for the major molasses treatment, it can be seen (Figure 3.2.3) that another fermentation stimulant is necessary - as in the case of the twice molasses and inoculant treatment (MMI). However, lactic acid as a proportion of **total** fermentation acids (Figure 3.2.2) indicates that MI is more effective; but neither are as effective as the major molasses treatment alone (M). The Forager inoculant (I) and amylase (AM) treatments gave increased amounts of lactic acid and also a higher ratio of lactic to total fermentation acids compared to the control, which illustrates that they are indeed effective fermentation stimulants. The high rate Add-Safe (ASAS), as an acidic fermentation inhibitor, reduced lactic acid content to a similar level as the content of the fresh, frozen grain.

Figure 3.2.4 shows that the 100% Sylade treatment reduced acetic acid more than 100% Grintona. This was also true for the 50% treatments, although the difference was not as marked. Both these major treatments contained much lower levels of acetic acid than Add-Safe, the only other acid fermentation inhibitor used. The fresh, frozen grain (FF), contained more acetic than lactic acid but after ensilation, the untreated grain (U) contained over twice as much lactic as acetic. The use of the double strength molasses treatments (MM & MMI) led to increased acetic acid contents compared with all the other treatments. The extra financial expense involved with the twice strength molasses, and the molasses and inoculant, is evidently unjustified.

In a good quality grass silage, acid production should be allowed to dominate alcohol (McDonald, 1981). In this experiment,

treatment SS appears unsatisfactory according to this criterion (Figure 3.2.5), with a very low acid to alcohol ratio (0.16). This ratio and that for treatment GG however, must be considered in the context of the severely restricted fermentation brought about by these treatments. Molasses showed a similar acid: alcohol ratio to Graintona 100%. The untreated barley grain with an acid: alcohol ratio of 2.3 represents a satisfactory fermentation. In the case of the minor treatments, the high strength Add-Safe (ASAS) had by far the greatest acid: alcohol ratio, with the frozen grain sample (FF) also showing an acidic content.

When the acid to alcohol ratios are viewed with the alcohol contents of the treatments (Figure 3.2.6), the 100% Graintona gives a sample with almost one third (3.6 g/kg FW) the ethanol content, compared to the 100% Sylade (10.8 g/kg FW). 50% Graintona also proved to be more effective in reducing an alcoholic fermentation than 50% Sylade. The untreated grain had a relatively low ethanol content, whereas the molasses promoted an ethanolic fermentation. The minor treatments involving molasses also showed a strongly ethanolic fermentation. Again, the high strength molasses, together with molasses combined with the Forager bacterial inoculant would not be recommended. These results substantiate the olfactory observations which were made during the opening of the pot silos, where a highly alcoholic/ fruity smell was noticed with all treatments involving molasses. The amylase and Forager inoculant also promoted a high ethanolic fermentation. The high Add-Safe and fresh, frozen barley grain samples showed low ethanolic contents, the latter indicating that alcohol producing bacteria are not initially dominant in the natural state.

The detailed water extract analyses have shown that Graintona, used at the commercially recommended rate, is an effective fermentation inhibitor for ensiled grain and that a combination of additives would not be justified. Sylade also, whilst acting as a fermentation inhibitor, does not reduce alcohol production to the same extent. Add-Safe used at the high level is not as effective as Graintona or Sylade in reducing lactic and acetic acids.

The aerobic stability experiment, designed to give a quantitative measure of the rate of moulding of the grain, did not substantiate the observations made, both when analysing the pot silos and when conducting the feed trial. Analysis was made of both the white mould and blue mould forming in the tubes. The former is less serious and grew in varying amounts on all the treatments, after they had been exposed to the air for a number of days. The latter is a *Penicillium* mould (*P. cyclopium*) and represents a greater danger in a feed (Hyde & Oxley, 1960; Stenwig & Liven, 1988; Rotter, 1989a). The initial moulding rates for white mould were very similar up to around nine days (Figure 3.3.1). From the results, it can be seen clearly that the 100% treatments were most effective in preventing white mould growth. However, growth continued at a slower rate in the 50%, molasses and control grain samples. Molasses appeared to be the most susceptible to white mould and this was also found during the feeding trial experiment. Surprisingly, the untreated grain sample showed less white mould development than the 50% acid grain treatments.

In the case of the blue mould, the initial mould appearance rates were much more variable (Figure 3.3.2). However, it is immediately apparent that negligible amounts of blue mould

appeared with molasses in the experiment and this was observed throughout the research with other similar samples deliberately left to mould. The untreated grain sample showed the greatest initial blue mould development, followed by the 50% and 100% Graintona treatments. The Sylade treatments then follow, the 100% developing less blue mould than the 50%. This pattern of difference was not observed in any of the grain containers, especially the large feeding trial barrels where, on opening, in the centre of the barrel, a clump of blue mould had already formed for both the 100% treatments (GG & SS). However, for the 50% treatments (G & S), no such mould growth occurred in the barrels.

It has already been stated that the initial rate of mould development is of greatest importance, as in practice, grain removed from a silo would not be left exposed to air for more than a few days. The experimental duration of 64 days did prove to be of interest, because even under these extreme conditions, the two 100% additives gave the greatest mould suppression. A possible source of error could be that the tubes had dried out. This would affect the 100% treatments and the untreated grain samples, which were of greater initial dry matter content (Table 3.1). Although the tubes had a dry plug at the top, on emptying them at the end of the trial, the grains were still moist further down the tubes, although they were in some state of decay. The lower degree of moulding for the 100% treatments, in this experiment, did not match all the other observations made throughout the study. In the case of the large barrels, pot silos and pH pots, the degree of mould development and pH values were very similar and showed the 100% treatments (GG & SS) to be the least effective for mould suppression. Each point on Figures

3.3.1 and 3.3.2 is the mean of three tubes, and the measurements made on the individual tubes never varied by more than 2cm in a particular triplicate set. It can only be suggested, as this experiment was performed towards the end of the feeding trial, that the grain had stabilized to the air, as the barrels had been opened on several occasions by this time and perhaps the initial moulding rate was not fully representative. It was unfortunate that time was not available for this experiment to be repeated. On the other hand, the experiment could be viewed as a recommendation for using the commercial additives at their advised rates. An appropriate comment made by the supervisor was 'one should never attempt quantitative experiments of visual observations'!

The buffering capacity of plants, or their ability to resist pH change, is an important factor in ensilage. Virtanen, in developing the A.I.V process (Section 1.1), was aware of this fact and he devised a rapid method for determining the amount of hydrochloric acid which must be added to a fresh crop in order to obtain the desired pH value. Interest is usually in the pH 4 to pH 6 range, as most plant materials, after maceration, have pH values around 6 whereas the pH of a well-preserved silage is about 4. The buffering capacity of untreated Atem barley was determined as 175 meq of 1 molar sodium hydroxide per kg DM. This placed the grain in the lower third of a population of 105 grasses analysed at this centre. Therefore, buffering capacity would not be expected to present a serious limitation to the achievement of a sufficiently low pH for effective preservation. The grasses gave values from 109 to 493 meq/ kgDM. Legumes are more highly buffered than grasses, giving results of >500 meq/ kgDM.

4.2 Experiment 2: Dacron bag incubations.

Table 3.2 clearly shows the very highly significant effects of time, treatment and the interaction of time and treatment for nitrogen disappearance *in sacco*. Reference to the individual results and to Figure 3.4 demonstrates how the full strength treatments significantly reduced rumen degradability, the Graintona additive being the most effective in protecting the grain protein. Indeed at the initial time '0' incubation, the 100% Graintona treatment was clearly the most effective, reducing nitrogen loss by almost ten percent compared to 100% Sylade, implying the grain protein would be much less soluble in the rumen environment. Figure 3.4 also shows that the 50% treatments are much less effective in protecting the grain protein. The molasses proved an effective control treatment in this experiment, giving an instantaneous *in sacco* protein loss of almost 80%. The protein protection effect diminishes with time, until after 48 hours, there are no significant differences in protection between treatments, although reference to the figures shows the full strength Graintona treatment was still superior. For a sheep given chopped hay, as in this experiment, compared to long hay, rumination time may be reduced from 9 to 5 hours. When only concentrates are fed, rumination is reduced to 2.5 hours (Swenson, 1984). The form of the degradation curve up to about 24 hours is most important, as this determines the proportion of the nutrient likely to escape rumen degradation under normal outflow rates. Applying this to the curve for the full strength Graintona, around 30% of the protein would still be undegraded after 10 hours.

When the constants *a*, *b* & *c* were obtained (Section 2.5) and the fitted curve drawn (Figure 3.4), the percentage

disappearance, p , can be related to the rate of passage of digesta from the rumen. The true or 'effective degradability', P , can be calculated from the equation: $P = a + bc / (c + k)$, where k is the outflow rate from the rumen per hour, expressed as a decimal fraction. The above equation was used to determine the effective degradability for nitrogen, organic matter and protein-free organic matter at two distinct rates of passage:- 4%/ hour ($k = 0.04$) and 8%/ hour ($k = 0.08$). The lower rate, 4%/ hour, is more applicable to sheep, whereas the higher rate is used for a ruminant under intensive production systems, such as the high yielding dairy cow. The mean effective degradability figures (Table 3.2), show that 100% Graintona (GG) is the best treatment for protecting high moisture barley grains from rumen degradation. The proportion of grain nitrogen reaching the duodenum would be doubled, compared with the molasses treatment (22% & 28% vs 10% & 12%, at outflow rates of 4% & 8% respectively).

Table 3.3 gives the analysis of variance for the *in sacco* organic matter disappearance. Here the individual sheep data were used, rather than the means from three sheep. Very highly significant effects ($P < 0.001$) were observed for individual sheep and there was a highly significant variation between periods ($P < 0.01$). However, the sheep/ treatment interaction was not significant. Figure 3.5 shows a similar pattern to the nitrogen degradability curves, but the effect of protection of organic matter is not as great as that for protein (Figure 3.4). It was interesting to note that the 100% Sylade curve intersects that of the 100% Graintona after approximately 5 hours of rumen incubation of high moisture barley, although the difference between curves was not significant, except in the 24 hour region.

The reversal of the half strength curves is also of interest. The 50% Graintona allowed a greater barley grain organic matter disappearance than the 50% Sylade treatment, which was significant until 16 hours of rumen incubation. In fact, the 50% Graintona was very similar to the molasses control, as can be seen from the figure and the superscripts in Table 3.3. Here, 50% Graintona showed even greater rumen degradability than the molasses control treatment in the initial stages of incubation. The similarities of the GG and SS full strength, and also the G and M treatments are distinctly visible in the calculated mean effective degradability figures. Obviously, using the Graintona additive at less than the commercially recommended rate would not give satisfactory results.

Protein-free organic matter (PFOM), was devised in this experiment to represent the starch content of the barley grains. Earlier enzymic methods of starch assessment (Clegg, 1956; MacRae & Armstrong, 1968) at this centre gave poor repeatability (J. Weir, personal communication). As the majority of the storage component of barley grain is starch (Waldo, 1973; Rasmusson, 1985), PFOM represents a quicker and more effective method of determining the amount of starch present in a large number of barley grain samples. Any mineral content of the grain samples is also taken into account, hence PFOM is a more accurate method than the protein-free dry matter used by Hyslop *et al.* (1989). The *in sacco* PFOM disappearances showed very highly significant ($P < 0.001$) effects for treatment, time and the treatment/ time interaction (Table 3.4). A significant period effect was also found. Graintona, used at full strength (GG), proved to be the most effective treatment for preventing the ruminal degradation of starch. In this case, 28% and 33% of PFOM would reach the

duodenum compared with 21% and 25% for molasses, at the respective outflow rates of 4% and 8%. Figure 3.6 shows a similar pattern to that of the organic matter digestion curves, although the difference between the two 100% treatments is more noticeable. As the main objective of the preservative is to protect protein and starch from rumen degradation, with subsequent release in the small intestine, an ideal digestion curve would be one with a steep initial gradient, which, after around ten hours, became less steep, almost forming a plateau. The steep initial gradient of the digestion curve would signify ruminal protection, where the shallower portion would indicate that no more protection could be afforded. Therefore, in order to fulfil the above criteria, a commercial additive would be required to demonstrate a degradation curve similar to that shown by the 100% Graintona treatment in Figure 3.6. It is crucial that the starch and protein are not overprotected as they would then be unavailable for further ruminant utilization in the small intestine.

The commercial grain preservative 'Graintona Plus' had the greatest effect on the *in sacco* protection of barley grain protein, since the principal effect of formaldehyde, on which Graintona is based, is to protect dietary protein from rumen degradation (Offer *et al.*, 1971; Barry, 1976). As Armstrong (1972) has shown, in cereal grains such as barley, starch exists as granules encapsulated in a protein matrix. Hence the rate of starch degradation in the rumen would also be reduced. The results of this experiment are in agreement with those of previous researchers who have shown that treatment with a formaldehyde additive will protect the barley starch and protein; thus reducing both their rates of degradation in the rumen

(Kassem et al., 1987; Van Ramshorst & Thomas, 1988; Hyslop et al., 1989; Morgan et al., 1989; McAllister et al., 1990a).

`Scanning electron microscopy photos in this study indicate that the reduced microbial digestion of starch is mainly due to protein "protection" rather than to the formation of "cross-links" in starch. The reduced digestibility of the protein matrix inhibits the access of bacteria to underlying starch granules. In [formaldehyde] treated barley, rumen bacteria gain access to underlying starch granules primarily by digesting small starch granules that lie on the surface of endosperm cells, rather than digesting away the surrounding protein matrix. As this process continues, more starch granules are removed and an intact protein matrix is left behind.'

[McAllister et al., 1990a]

4.3 Experiment 3: The feeding trial.

`Differences between sheep were responsible for much of the variance in these parameters.'

[Faichney & White, 1977]

`In addition to differences between animals, there are differences in single animals at different times.'

[Hungate, 1966]

The sheep hay dry matter intakes (HDMI) showed a large amount of individual variability and gave no significant effect for treatment. In an attempt to reduce the variability and relate the figures more closely to the individual sheep, the daily HDMI for each sheep were divided by their mean live weight over the three periods. It was hoped that this would reduce the between sheep variability and that any effects observed could be attributed to treatment. Table 3.5.1 shows the analysis of variance for the first five day sessions of the three, 20 day periods. Even after moderation of the results, the effects of treatment proved to be non significant. The coefficient of variation was also quite large (10.90%). A possible advantage of treatments aimed at reducing grain degradation is the achievement of a better rumen environment, with a more stable, higher pH and larger numbers and

activity of cellulolytic bacteria. This would promote more rapid hay digestion in the rumen, increased ruminal emptying rate and hence stimulate intake. Therefore, the daily HDMI should have been greatest in the Graintona and Sylade treatments, with the potential for increased levels of animal production. Table 3.5.1 shows that the additives did not stimulate intake, with the control diet giving the highest corrected hay intake (18.51 g/kgLW), followed by the molasses (18.44 g/kgLW). These two diets also show an increased corrected mean. This would indicate that if all ten sheep had tested the diet, the hay intake over the first 5 days of each period would have been even greater. A further point, which also applies to Table 3.5.2, is that the corrected mean for the first three treatments, Graintona 100%, 50% and 100% Sylade is always reduced; whereas that for the second three treatments, 50% Sylade, molasses and the control grain is increased. Table 3.5.2 shows the results for days 6-10 of the *ad libitum* hay intake over the three periods. This is of greater interest, as the sheep had 5 days to become settled into the new period and accustomed to their new diet. However, no significant effects were seen for sheep or treatment in this session of each period and the coefficient of variation was larger than the day 1-5 session. Differences between individual treatments are not as marked and the mean daily hay intake has increased for each treatment in comparison with Table 3.5.1.

When hay was restricted to 500g FW for ten days, Table 3.5.3 shows that the control treatment prompted the biggest hay intake. 100% Sylade followed, with the 100% Graintona treatment giving the third highest mean HDMI. The coefficient of variation is also reduced in this restricted period (6.91%).

No significant effect of treatment was found on the

percentage organic matter digestibility of the whole diet, with all treatments being over 70% digestible (Table 3.6). Sylade 100%, closely followed by the control treatment promote the greatest whole diet OMD. 100% Graintona again is in third place. Faichney and White (1977a), in studies with sheep, found that formaldehyde treatment of barley/ soya bean diets increased the digestion of protein in the small intestine, but did not significantly affect the digestion of organic matter in the whole gastro-intestinal tract, or its partition between the stomach and the intestines.

As Experiment 2 has proved, the two formaldehyde-based additives significantly decreased the ruminal dry matter degradation of starch and protein. Therefore, it could be assumed that with these two treatments, rumen conditions would be more favourable for forage digestion, with reduced incidence of ruminitis, improved microbial yields and improved efficiency of energy use in the animal (Rode et al., 1986). Also, it could be speculated that the increased ruminal forage digestion offered by these two additives would promote increased HDMI (Orskov & Fraser, 1975; Kassem et al., 1987; Hyslop et al., 1989). It was unfortunate that this was not observed in the feeding trial, given the amount of time and care taken, and rather surprising that the results were inconsistent.

In hindsight, it could be stated that the feeding of the young sheep with high moisture barley was carried out with too much caution. Orskov (1979) recommended a concentrate supplementation rate for sheep of $50 \text{ g/kg}^{0.75}$ per day of mature, dry barley. Given the moisture content of the barley used in the trial (451 g/kg), a 700g meal per day would be providing a daily intake of around 384g dry matter. This would vary slightly,

depending on the treatment (Table 3.1). For a 35kg sheep, the Orskov rate of $50 \text{ g/kg}^{0.75}$ suggests a daily supplementation of approximately 720g of fresh barley, with a moisture content in the region of 150 g/kg; an average figure for barley at normal harvest time (SAC, 1989). This equates to 612 gDM per day and compared to 384 gDM per day, it would appear that the sheep were only receiving 63% of the rate recommended by Orskov. However, in this experiment the moist barley was given as one single meal per day. The forage to concentrate ratio for the *ad libitum* session was 70:30 (DM) and for the restricted session was almost 50:50 (DM). The former is identical to that used by Hyslop et al. (1989), who observed a 14% increase in hay intake with rolled barley treated with formaldehyde-based 'Sylade 2'. Although the sheep were prepared for the trial with 'Propcorn' moist barley and then with the actual Atem barley to be used (Section 2.6), it was well recognized that the 9 month old sheep were unaccustomed to barley of a high moisture content. Given the enhanced palatability of the feed (Krall, 1969; FSL Bells, 1989), extreme caution was exercised to prevent any risk of illness amongst the young sheep and a moderate barley feeding level was adopted, as they were to be immediately designated for a following experimental feeding trial. Also, due to the paucity of relevant research data qualifying actual feeding rates to ruminants for high moisture barley, it was decided to err on the side of caution and give smaller meals. Clearly, the dry matter content of the feed must be taken into account when devising a ration. Towards the end of the feeding trial, it was noted that the sheep appeared quite restless and hungry, as in this study, feeding levels were not adjusted to take account of live weight gains (Orskov, 1969; Orskov & Fraser, 1975; Van Ramshorst & Thomas,

1988). At the end of the *ad libitum* stages of period 3, the hay refusals were much reduced and in the case of the molasses treatment, all of the hay was consumed by Sheep 9 on two successive days. The incomplete block design used and on which this feeding trial depended, added a further complication to achieving significant results. Here, the treatment differences are confounded to the extent of 20% by differences between sheep. This means that up to one fifth of the variability between treatments could be due to animal effects. This was a limitation of the design used. The effects of freezing the control grain for storage and of the moulding of the 100% treatments on achieving satisfactory results are open to question.

A possible explanation for the results obtained is that the sheep given the unprotected treatments, even at nine months old, might have been able to sense the unfavourable rumen conditions which it was assumed would be caused. It is well known and generally accepted that animals can regulate their food intake (Snapp, 1962; Baile & Forbes, 1974; De Jong, 1985) and, as individuals, can reach satiation at different dry matter intakes. Furthermore, Graitona is specifically designed to provide a satisfying meal (FSL Bells, 1989) and the sheep on these treatments could have reached their set point earlier. Also, the 100% Graitona and Sylade had the highest dry matter content of the treatments (Table 3.1). The charts in Appendix E give the individual hay dry matter intakes for each sheep in each of the three periods, during days 6-10. The increased intakes from period 1 through to 3 can be seen immediately. All sheep were housed in identical conditions (Section 2.6). Variabilities in individual intakes can be seen in Period 1 (Appendix E1), for example, the 100% and 50% Sylade treatments (SS & S). Period 2

(Appendix E2) shows an individual intake variation of over 400g between sheep on the 50% Sylade treatment (S), on day 10. An initial glance at the data for Period 3 (Appendix E3) might suggest that the initial postulate had been proved, with visibly higher intakes for the treated samples than the molasses and one of the untreated ones. Without doubt, these charts certainly support the quotations given at the beginning of the discussion of this experiment.

Another possible explanation for the formaldehyde treatment effect not being found with the young sheep as compared to cattle is the way in which sheep ingest their food. It is well known that sheep nibble and chew their food much more thoroughly than cattle. Prolonged chewing of the crimped grains could cause a disruption of the protein matrix, possibly even breaking it up completely. In this case, the ruminal protection of protein and starch would be much reduced and no effect on HDMI would be seen. In this context, it should be remembered that the protection observed *in sacco* was for grains that had not been ground or chewed prior to rumen incubation. The processing of the feed is crucial if it is to be effectively utilized by the ruminant (Section 1.6). With finely milled grain, one would not expect an effect on protein and starch protection with formaldehyde, as the whole of the endosperm is exposed; although this was observed by Hyslop *et al.* (1989). Therefore, a method which is sufficient only to crack the grain is preferred (Armstrong, 1972; Hale, 1973; Orskov & Fraser, 1975; Orskov, 1979; 1987; Owens, 1986). 'Coarse rolling' would not break open all the hulls of the cereal grains. Increased pressure on the grain rollers would result in a 'medium rolling', which would ensure that all the kernels are broken to expose the endosperm

and this is very similar to the crimping used in this research. Studies in Canada using these three degrees of processing have shown that the greatest ruminal protection of protein and starch due to formaldehyde treatment occurred with the medium rolling of the grains (T.A. McAllister, personal communication).

The results of this research have been insufficient to show that formaldehyde protection of grain starch and protein stimulated hay intake under the conditions used. Nevertheless, they do in themselves raise a number of interesting questions in the areas of feed intake and rumen metabolism and are worthy of further investigation. Had the sheep used been fistulated, then rumen pH samples could have been obtained, which might have given a much deeper insight into the rumen conditions caused by the various treatments. As previously stated, the sheep were not fed fully to appetite. They received only one meal for the whole day and this was rapidly consumed. This method of feeding may be considered as extreme in comparison with normal ruminant feeding systems. It may be postulated from the results of this study that the rumen environment of the young sheep was not altered sufficiently to give an effect on hay dry matter intake and, that in this growing, fattening trial, the resultant effect of formaldehyde protection did not manifest itself in practical terms. Any depression of rumen digestibility in the control or molasses treated diets could have been compensated for in the lower gastro-intestinal tract. Even on the control diet, rumen conditions in the sheep cannot have varied significantly from the norm. Therefore, it can be speculated that formaldehyde protection of barley protein and starch would be most appropriate for animals under high production conditions, where a modified rumen environment is likely to be beneficial.

4.4 General Discussion.

The three experiments involving Preservation Studies, the Dacron Bag Incubations and the Feeding Trial have been discussed in detail and it now seems appropriate to consider the research overall and the future implications for High Moisture Barley.

The Atem barley with a 549 g/kg dry matter content used in this research was subjected to two forms of processing; a physical one of crimping and a chemical one by the inclusion of the 'Graintona Plus' additive, in order to obtain maximal results for grain preservation and feed efficiency with ruminants. Orskov et al. (1970), Spicer et al. (1986) and Theurer (1986) hypothesized that processing probably enhances energy and nitrogen economy for an animal by minimizing starch fermentation and protein synthesis. Also, Owens et al. (1986) suggested that,

'Processing methods to reduce particle size or alter the protein matrix which cement starch granules together will increase the extent of digestion both in the rumen and small intestine.'

Atem, the two-rowed genetic variety of barley used is a long-strawed, large-grained variety, originally from the Netherlands. It is recommended for both animal feed and malting. It is well suited environmentally to climatic conditions which prevail in areas such as the West of Scotland.

To store high moisture barley by the method of ensilation, the main requirement is an effective, inexpensive additive, combined with meticulous ensiling techniques to give complete preservation, which results in restricted mould growth and good aerobic stability once the clamp is exposed. One of the most common mistakes is to ensile the barley with kernels that are too dry (Fletcher, 1988).

A quantitative assessment of the amount of mould developed has not been considered in this experimental work. With the material

which appeared, it would be possible to furnish data for a further microbiological research study. A quantification of the mould could be made with the Fungal Contamination Index recently devised by Rotter et al. (1989b). As already stated in the literature (Section 1.8.1), knowledge of the fungal content of grain aids considerably in the formulation of feed and also helps to reduce costly losses of both grain and animals. The distribution of *Penicillium* mould in the barley demonstrated in this investigation is consistent with the findings by Hill and Lacey (1984) in UK barley and Stenwig and Liven (1988). It is suggested that the moulding which occurred could not be attributed to the ensilation process. Hyde and Oxley (1960) hypothesized from the results of their investigations that organisms associated with the grain are not all killed by prolonged airtight storage at high moisture content, but some remain. The mould which occurred in this research may have arisen from surviving micro-organisms, or chance contaminants picked up as the grain was being run through the crimper. It may have been due to moisture migration from the centres of the containers as suggested by Gibson et al. (1988) or it may be related to the genetic characteristics of the Atem variety of barley used. In this research, the storage was for a period of at least 97 days, whereas the storage time used by other researchers has varied from a small period of seven days to as long as two years. Length of storage time is another interesting contributory factor to consider.

The resultant feed from the ensiled high moisture barley should be consumed at a sensible rate and not left exposed to air for prolonged periods of time. In this experimental research, it was necessary, on occasions, to weigh out quantities of feed in

advance, due to set organizational factors outside personal control. This pre-preparation would test all preservatives to the utmost and would not be encountered in a normal physical commercial environment. If the high moisture barley was used on a commercial scale, for example, with 30 lactating cows each consuming about 10kg/ head/ day and assuming this was given to the animals in two feeds, then 150kg would be removed from the clamp face for each feed. With amounts in this region being removed and if the silage clamp was constructed long and narrow as recommended (Krall, 1969; FSL Bells, 1989), aerobic stability would not prove to be a major problem. In similar commercial trials with 'Graintona Plus', losses due to moulding have been negligible (M. Lewis, personal communication). Compared to some of the values used by other researchers in the literature, the Atem variety of barley used in this research, at 451 g/kg moisture (45%), was very moist. Although the Atem barley was of relatively low dry matter content compared to other varieties observed and handled, it was very friable and did not clump together when pressed, which is the standard test for high moisture barley (Fletcher, 1988). The free flowing characteristics of Atem, even at the high moisture level, would not present a problem if the grain was designated for use in an automated feeding system or a movable manger.

Animals of all ages, especially ruminants, will sometimes react unfavourably to radical changes in their feed, no matter how scientifically well designed or beneficial the new feed may be. As many researchers have reported throughout the literature, this has proved not to be the case with high moisture grain, which is highly palatable and well accepted by all animals (Krall, 1969; Jones et al., 1974; Perry, 1980; FSL Bells, 1989).

Acceptability of the grain did not present a problem in this experimental feeding trial. It has been suggested by some feed specialists that if the user is in any doubt about how the high moisture barley will be accepted, then it should be introduced gradually into the feed ration.

The young sheep used in this feeding trial (Section 4.3) followed the pattern set by the sheep referred to in the literature, because they too were individuals. As individuals, they reached their own point of satiety as the histograms in Appendix E illustrate. Individual characteristics determined feed intake and individual differences occurred. Regardless of the fact that the barley contained *Penicillium* blue mould it was consumed rapidly. It must be emphasized that the whole meal was given only once a day at 08:45 hours and in all cases was consumed by 10:00 hours. Although fed only maintenance rations, the young sheep did make live weight gains. It could be speculated that if they had been fed at commercial production levels, they would have reached an early finishing grade, as found by: Dinusson et al. (1964); Krall (1969); Perry (1980); Laksessvela (1981); Kennelly et al. (1988b) and Mathison et al. (1988; 1989).

'comparison of sites of digestion at two levels of intake has demonstrated that the results obtained and their interpretation will depend upon the level of feed intake used. It is important to stress that digestion studies made at *ad libitum* levels of feeding are more likely to aid interpretation of results of growth studies from cattle fed *ad libitum* than similar digestion studies at restricted intakes....Had the digestion study [of formaldehyde-treated soya bean meal] been conducted at restricted intakes only, these responses would have been much less clear.'

[Lindsay et al., 1983]

It has been well established that the rapid fermentation of barley in the rumen often causes digestive disturbances, such as

acidosis, ruminitis, abomasal displacements and liver abscesses (McDonald & Warner, 1975; Orskov, 1986). High moisture grain does not present these problems (Krall, 1969; Perry, 1980; McKnight et al., 1973; Laksesvela, 1981; FSL Bells, 1989). No visible clinical symptoms of acidosis or illness occurred amongst the sheep used in this research. The reduction of ruminal starch digestion reduces the occurrence of digestive disturbances and results in a rumen pH which is more favourable for fibre digestion in ruminants fed mixed diets of concentrates and forage. McAllister et al. (1990a) postulated that,

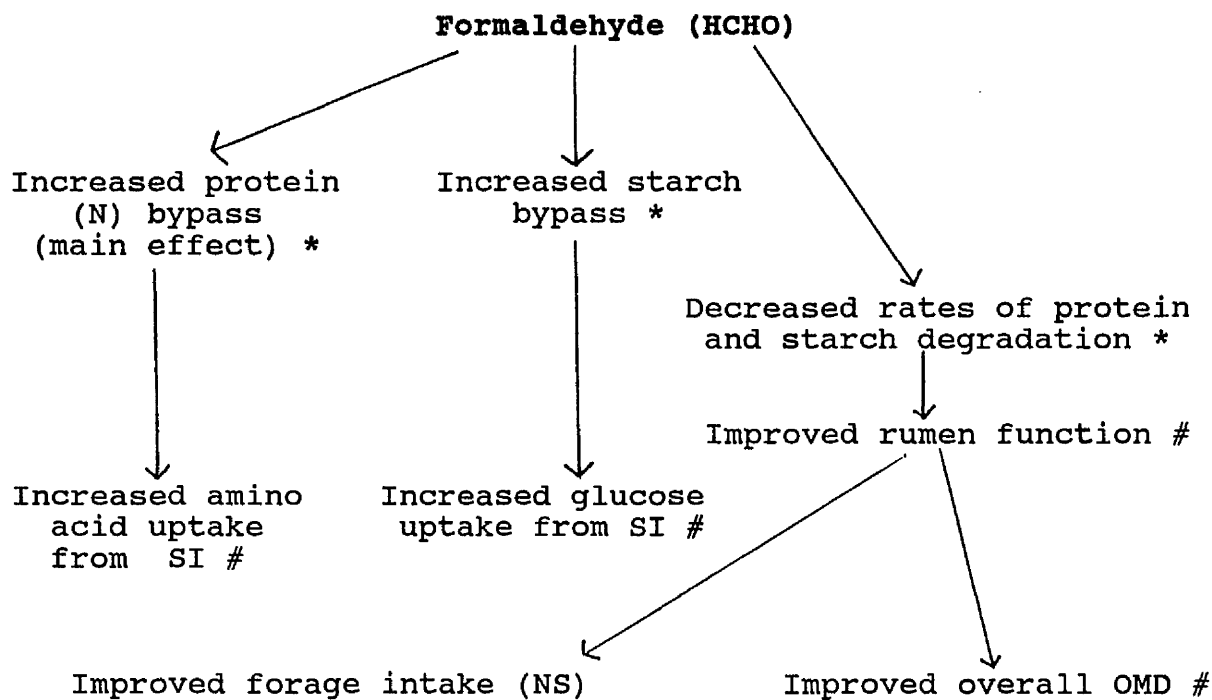
'Economic losses due to these disturbances would be reduced if the rate of microbial starch digestion could be controlled.'

This research has demonstrated that 'Graintona Plus' (formaldehyde) chemical treatment of high moisture barley successfully reduced rates of grain degradation by rumen micro-organisms. The results of Experiment 2 (Section 4.2) are in agreement with those of Kassem et al. (1987). In their research using lactating cows, they showed that the treatment of rolled barley with an acidified formaldehyde additive effectively reduced *in sacco* disappearance of barley starch and protein. In feeding trials with dairy cows conducted by Kassem et al. (1987), comparisons of untreated and formaldehyde-treated barley, given as supplements to grass silage diets, showed that treated cereals increased milk yield and protein content and tended to reduce milk fat content. In addition, Van Ramshorst and Thomas (1988) found that formaldehyde treatment of barley reduced rumen degradability and increased the amount of both starch and protein available for digestion in the small intestine of sheep. Owens et al. (1986), from their research and performance data on growing cattle postulated that 42% more energy was available for the

ruminant; from the 47% to 88% of starch digested in the small intestine and the 33% to 62% of starch digested in the large intestine. Recent studies of McAllister et al. (1990a) indicated that formaldehyde treatment of barley caused a delay in the digestion of barley starch for up to 24 hours.

In this research, degradability of protein and starch was significantly lowered by the treatments and 'GrainTona Plus' proved to be the most effective. These results would imply an improved rumen environment, containing a larger amount of cellulolytic bacteria giving a more rapid fibre digestion and improved forage intake. This benefit was not observed in the present study using a moderate hay to barley ratio of 70:30 (DM basis). A response should only be anticipated at high grain intakes, when the unprotected starch induces significant rumen acidosis. The observed reduction in degradability of starch and protein suggests also that greater amounts would bypass the rumen to be more efficiently utilized in the small intestine for production purposes than VFA absorbed from the rumen. This would increase animal performance and improve milk lactose synthesis, as glucose would not have to be formed through the recognized gluconeogenic pathway. A summary of the potential benefits of formaldehyde treatment of barley grain is presented in Figure 4.1. The effects observed by Kassem et al. (1987) were mainly due to increased levels of protein entering the small intestine. The greatest effect of formaldehyde treatment is maintaining the integrity of the protein matrix (McAllister et al., 1990a) which was also seen in this research (Section 4.2).

Figure 4.1 Possible effects of formaldehyde treatment of grains.



* Shown in this research.

Not recorded.

NS Not significant.

SI Small intestine.

Methane production can also be reduced by the use of formaldehyde-treated grain (J.G. Buchanan-Smith, personal communication). Approximately 5g of methane is formed for every 100g of carbohydrate digested and the ruminant loses about 12% of the digestible energy of its food in the form of methane.

Future Implications for High Moisture Barley.

With the ever increasing legislation affecting agricultural practices, High Moisture Barley could have an important place in the current trend for organic production technology. As outlined in the literature (Section 1.7), the high nutrient content of barley is achieved naturally. Early harvesting reduces the need for the input of nitrates to the crop and together with this

unique method of ensilation, eliminates the need for pesticides and herbicides. These are not required because, with early harvesting, shattering of the weed seeds does not occur. Feed costs are the largest single expense in animal production. These costs can be reduced by the inclusion of High Moisture Barley in farming production systems. Labour, machinery, transport, handling costs and rate of deterioration are major factors to be considered in choosing an alternative feed. A reduction in feed costs that still maintains animal performance has a significant effect on gross profit margins, for both grain and livestock producers. Self-sufficiency and diversification to reduce risks are important factors to consider and the production of home grown cereals can result in a number of economic advantages.

The free trade market of Europe 1992 will probably bring about changes in feeding and breeding policies, with a much greater emphasis on efficiency and quality. More consideration will have to be given to health conscious consumer options. Future trends and payments will be for the production of better quality meat and milk with an increased protein to fat ratio. This can be achieved with the use of 'protected' grain together with an increase in lactose content whilst still maintaining acceptable VFA levels (D.G. Armstrong, personal communication). Payment for milk protein is already in operation. Also in the future, new types of additives will have to be developed to replace the use of formaldehyde, which under the COSHH Regulations (1988) is not classified for use on farms (C. Shorrocks, FSL Bells; personal communication). Predicting the protein requirements of ruminants and the manipulation of the rumen fermentation is now generally considered to offer a new means of improving the efficiency of feed utilization and the

new, safer additives are awaited with great interest. In the US, feedlot systems have become very efficient and livestock producers are able to predict exactly the daily growth rates of animals.

Further research and experimental work on High Moisture Barley and additives is required before the effects of treatment on animal performance can be evaluated in relation to economic terms. As well as being concerned with finding solutions to problems of production, researchers also need to be concerned with the health and physiological parameters of ruminants. Research on the sensations of hunger and appetite, factors influencing feed intake and the effect of the environment outside the ruminant are all important areas which would provide interesting and valuable information. In 1966, Hungate suggested that,

'the factors controlling appetite are extremely complex, but their elucidation could be extremely important in achieving maximum productivity'

and this statement is still applicable today. The traditions and customs of people control the consumption of barley as food for humans or for animal feed. It has become increasingly necessary to consider the feed quality of barley to be important and this consideration must be continued in order to meet the nutritional demands of the future. Cultivated barley (*Hordeum Vulgare* L.) already has the advantage over the other major cereal grains, because it is one of the most frequently used experimental plants in studies dealing with genetics, cytology, mutation induction, crop populations and breeding methods (Rasmusson, 1985) and its metabolizable energy can be utilized by the ruminant most efficiently (McAllister et al., 1990b).

PART FIVE.

CONCLUSION.

5.1 CONCLUSION.

From the results of this research study, it may be concluded that the successful storage of treated barley grain with a high moisture content is possible, providing meticulous care is taken throughout the whole ensilation process to achieve anaerobic conditions.

The resultant concentrate feed from the ensilation is highly palatable and well accepted by ruminants of all ages. Ensiled, High Moisture Barley may be given as the sole concentrate energy component in a diet, or included as part of a well balanced, mixed feed ration.

The commercial additives examined may be used successfully to achieve protection of both nitrogen and protein-free organic matter in barley cereal grain from rumen degradation. The structure and composition of cereal starches and their interaction with protein play a major role in the degradability and feeding value of grains for ruminants.

This research has demonstrated that the 'Graintona Plus' additive achieved, overall, the most desirable results and that it has the potential to be an economic grain preservative, when applied correctly, according to the manufacturer's instructions. The other additives were less effective when applied to the High Moisture Barley at rates other than those commercially recommended. No incidence of acidosis occurred, the sheep remained healthy throughout the experimental period and each one made live weight gains. The care which is necessitated and the effort involved during the preparation and preservation of the High Moisture Barley is well rewarded with an end product which is a quality concentrate feed.

Ensiled, High Moisture Barley is economically viable as a nutrient feed source, but the costs incurred will be variable in relation to the type of farming practice. The profitability of the process is dependent upon the cost of processing, the effective feeding value and the type of ruminant system in which the High Moisture Barley is used. The final results are dependent upon environmental factors, the genetic variety of the barley used and the type, age and characteristics of the animals involved.

At the present time there is a paucity of research data on the feeding value of ensiled, High Moisture Barley cereal grain for ruminants. More information is required for both animal and crop producers and barley itself, as one of the major cereal grains, merits further detailed study and investigation. Further research involving greater numbers of ruminants in feeding trials is needed to define, determine and establish more exact, accurate data and conclusive evidence on the nutritional value of High Moisture Barley as a concentrate food.

The most successful use of High Moisture Barley is in specific climatic regions, where there is an early return to field capacity and unreliable weather conditions, especially at the time of traditional harvesting. It has been clearly illustrated in recent years, that the pressures to increase the efficiencies of ruminant production are met, in the majority of cases, with the feeding of more concentrates; and future trends indicate that this is likely to continue. There are two specific situations where the use of ensiled, High Moisture Barley as a feed source would be ideal and of high beneficial value. The first is as a concentrate feed for high yielding dairy cattle and the second is as a concentrate feed for sheep supporting multiple

births; as these represent unnatural, artificial situations designed and demanded by man and with which the ruminant is expected to cope. As Orskov has stated -

`The ruminant animal is perfectly capable of manufacturing sufficient glucose for its normal requirements from propionate in the liver, as it has evolved in this fashion to exist on a herbivorous diet'

[personal communication, 1990].

APPENDICES.

APPENDIX A

Eslick - Hockett Classification of Maturation Stages of the Barley Kernel.

1. **Milk stage.** When the kernel is pinched in two, surface moisture is readily evident among the developing white starch granules without squeezing the half kernel. Typically the heads, leaves, and awns (beards) are green. The moisture percentage about 8 days before the start of the dough stage is estimated to be about 64%.

2. **Early dough stage.** When the kernel is cut in two there is no readily evident moisture but moisture is readily evident when the half kernel is slightly squeezed between the thumb and forefinger. Typically the heads, leaves and awns are green but usually with a tinge of yellow.

3. **Soft dough stage.** When the kernel is cut and heavy pressure applied, free moisture can be observed among the starch granules. Faint tinges of green are usually evident on the lemma (husk covering the back or rounded portion of the kernel). Typically the awns are yellow or reddish and the lower leaves are turning yellow.

4. **Dough stage.** Kernels readily cut with thumbnail, no free moisture can be observed when kernel is squeezed between thumb and forefinger, no wrinkles on lemma. Typically there is little or no green colour in the heads, most of the green colour is gone from the leaf blade but the leaf sheath (portion of leaf that is wrapped about stem) is usually green.

5. **Hard dough stage.** Kernel can be cut with thumbnail without excessive pressure. Kernels have a translucent or 'steely' appearance. The lemma covering the back of the kernel is not wrinkled. Typically nearly all of the green colour is gone from

the plant.

6. **Drying stage.** Kernels cannot be cut with the thumbnail. At least some kernels on the head are translucent or 'steely' in appearance. Very coarse wrinkles on the backs of at least some kernels in the head.

7. **Ripening stage.** No translucent or 'steely' appearing kernels. Kernels are finely wrinkled on the back. Heads are erect or not curved until a portion of the tip of the head is parallel with the stem.

8. **Over - ripe stage.** Heads are hanging until the tip of the head is pointed straight at the ground.

Taken from KRALL, J.L. (1969).

The composition of whole barley plants at different stages of growth*

	Heading completed (58)	Flowering (68)	Watery ripe (71)	Milky ripe (75)	Mealy ripe early (84)	Mealy ripe late (86)	Ripe for cutting (92)
A. Proximate constituents (mean of 8 cultivars)							
DM (g kg^{-1})	191	200	206	293	353	387	425
Crude protein (g kg^{-1} DM)	103	87	72	67	56	60	66
Ether extract (g kg^{-1} DM)	19	14	13	13	14	16	13
Crude fibre (g kg^{-1} DM)	313	321	286	253	204	195	254
Ash (g kg^{-1} DM)	75	65	56	47	38	35	35
WSC (g kg^{-1} DM)	169	180	249	318	242	147	46
D-value	62	54	58	61	63	61	55
B. Carbohydrate and organic acid components (c.v. Zephyr)							
Fructose (g kg^{-1} DM)	60	50	41	31	29	31	22
Glucose (g kg^{-1} DM)	60	60	42	29	28	20	11
Galactose (g kg^{-1} DM)	0	0	0	1.6	4.4	4.6	5.8
Sucrose (g kg^{-1} DM)	19.0	14.6	22.8	33.0	21.3	19.7	4.4
Oligosaccharides† (g kg^{-1} DM)	16.1	33.5	68.8	76.0	32.1	34.5	15.1
Fructans (g kg^{-1} DM)	30.8	32.5	72.0	128	122	66.0	23.0
Starch (g kg^{-1} DM)	2.8	2.5	3.8	10.2	18.5	34.8	41.3
Acetic acid (g kg^{-1} DM)	4.3	7.1	3.3	6.5	6.4	6.4	5.1
Malic acid (g kg^{-1} DM)	56.3	28.2	17.3	13.1	7.1	3.2	1.5
Citric acid (g kg^{-1} DM)	4.8	4.0	5.4	4.3	2.4	0.9	0.6
pH	5.9	5.9	5.9	5.9	5.9	6.0	6.0

* Numbers in parenthesis refer to Zadoks 2 digit code classification

† Excluding sucrose but including short-chain fructans

Taken from McDONALD (1981).

APPENDIX B

Method for Preparation and Analysis of Water Extracts.

Grain/ Silage VFA, Ethanol & Lactic Acid Determination (Updated DEC 89).

Reagents.

Name	Grade	Risk & Spillage Phrases	
Oxalic Acid	AR	R38	SP3
Pivalic Acid	Aldrich	Corrosive	SP3
Ethanol	Burroughs		SP1
Acetic Acid	AR	R35	SP1
Propionic Acid	AR	R34	SP1
Butyric Acid	AR	R34	SP3
Isobutyric Acid	AR	R21/22	SP3
Valeric Acid	AR	R34	SP3
Isovaleric Acid	AR	R36/38	SP3
Lactic Acid	AR	R34	SP3

Solutions Required.

1. Pivalic Acid Solution: 0.22g Pivalic acid per 100ml of distilled water.
2. Oxalic Acid Solution: 4.725g Oxalic acid per 500ml of distilled water.
3. Standard Solution :-

First make up 100ml stock solutions of each of the following compounds:

Compound	Approx. Wt. (mg/100ml)
Ethanol	500
Acetic acid	500
Propionic acid	500
Isobutyric acid	500
Butyric acid	500
Isovaleric acid	500
Lactic acid	5000

1. It is easier to measure out the approximate weight of compound (using a disposable pasteur pipette) and record the exact weight.
2. Once the stock lactic acid solution has been prepared transfer it to a 150ml Soxhlet flask and reflux for 24 hrs. Transfer the solution to a 100ml blue-capped bottle. This solution is now ready to be used in the mixed standard.

Preparation of the mixed standard.

Take 10ml aliquots of all these stock solutions into one 200ml volumetric flask, make up to the mark and mix well.

Calculation of the concentrations in the mixed standard.

The concentrations of all the compounds in the mixed standards are expressed as ppm or mg/L .

For all the compounds except lactic acid the concentration in the mixed standard is simply the number of milligrams of compound weighed into the stock standard divided by 2. For example if 512mg of ethanol had been used for the stock standard then the final concentration in the mixed standard would be 256 ppm.

In the case of lactic acid the concentration has to be calculated from the standardisation procedure.

Method: Pipette 10ml of the refluxed lactic acid stock solution into a 200ml volumetric flask make up to the mark and mix well. Titrate 50ml aliquots of this diluted lactic acid solution against 0.05M NaOH, using Phenolphthalein as the indicator. Carry out duplicate blank estimations.

The calculation is as follows:

$$\text{ppm Lactic acid (in mixed standard)} = (T-B) * 90.08$$

Where T = lactic acid titre and B = blank titre.

Preparation Of Extract.

1. Transfer 20g of fresh grain/ silage to a 250ml wide-necked bottle and add 100ml of distilled water.
2. Cap and shake mechanically for 1 hr.
3. Filter through a muslin or nylon cloth and collect the filtrate. This filtrate can now be stored in the deep freeze until required.

Procedure.

1. Take 5ml of the silage filtrate, effluent or standard using an automatic pipettor into a 12ml centrifuge tube.
2. Add 1ml of Pivalic Acid Solution and 4.0ml of Oxalic Acid Solution. Cap the tubes and mix well.
3. Centrifuge at 3000 r.p.m. (speed setting 7) in the refrigerated centrifuge (pre-cooled to 4°C) for 10 minutes.
4. Remove supernatant into an autosampler vial and cap. Samples are now ready for GC analysis.

Note.

The samples should be kept as cool as possible until they are about to be analysed.

G.C. Details.

AMS 93 Gas Chromatograph fitted with autosampler.
(Analytical Measuring Systems Ltd., Cambridge.)
Injection volume = 1 μ l. Oven temperature = 165°C.
Detector and injection temperatures = 200°C.

Column Details.

1.83m X 2mm internal diameter (glass) containing packing of
4% Carbowax 20M coated on Carbopack B-DA 80-120 mesh (Supelco
Inc.). Nitrogen flow rate = 24ml per minute.

Safety Notes.

1. Wear goggles and disposable gloves while measuring out the acids.
2. A spillage of lactic acid should be treated as follows:
Spread soda ash liberally over the spillage, mop up cautiously with plenty of water and run to waste.
3. All other spillages should be mopped up with plenty of water and run to waste down the sink.

APPENDIX C

Analyses of variance on the percentage waste and pH results for each treatment, obtained from the triplicated pot silos.

Analysis of variance on % waste.

SOURCE	DF	SS	MS	F	
TREAT	12	61.372	5.114	8.37 ***	(P < 0.001)
ERROR	26	15.880	0.611		
TOTAL	38	77.252			

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

CODE	MEAN	STDEV	
GG	1.3333	0.7095	(----*----)
G	0.2000	0.1732	(----*----)
SS	4.9333	2.5325	(----*----)
S	0.0000	0.0000	(----*----)
M	0.0000	0.0000	(----*----)
U	1.9667	0.5686	(----*----)
MM	0.4667	0.4163	(----*----)
AS	0.0000	0.0000	(----*----)
ASAS	1.1667	0.2517	(----*----)
MI	0.9667	0.3786	(----*----)
MMI	1.0333	0.5132	(----*----)
I	0.8667	0.1155	(----*----)
AM	0.8667	0.1155	(----*----)

0.0 2.0 4.0 6.0

SED = 0.638

Analysis of variance on pH.

SOURCE	DF	SS	MS	F	
TREAT	12	5.610785	0.467565	942.38 ***	(P < 0.001)
ERROR	13	0.006450	0.000496		
TOTAL	25	5.617235			

INDIVIDUAL 95 PCT CI'S FOR MEAN
BASED ON POOLED STDEV

CODE	MEAN	STDEV	
GG	5.3650	0.0354	*)
G	3.7600	0.0141	*)
SS	4.5800	0.0424	(*)
S	3.8000	0.0424	(*)
M	3.7100	0.0000	*)
U	3.6800	0.0000	(*)
MM	3.7900	0.0000	(*)
AS	3.9100	0.0000	*)
ASAS	3.8200	0.0141	*)
MI	3.7500	0.0000	(*)
MMI	3.6700	0.0283	*)
I	3.8000	0.0141	(*)
AM	3.7700	0.0141	*)

4.00 4.50 5.00

SED = 0.022

APPENDIX D

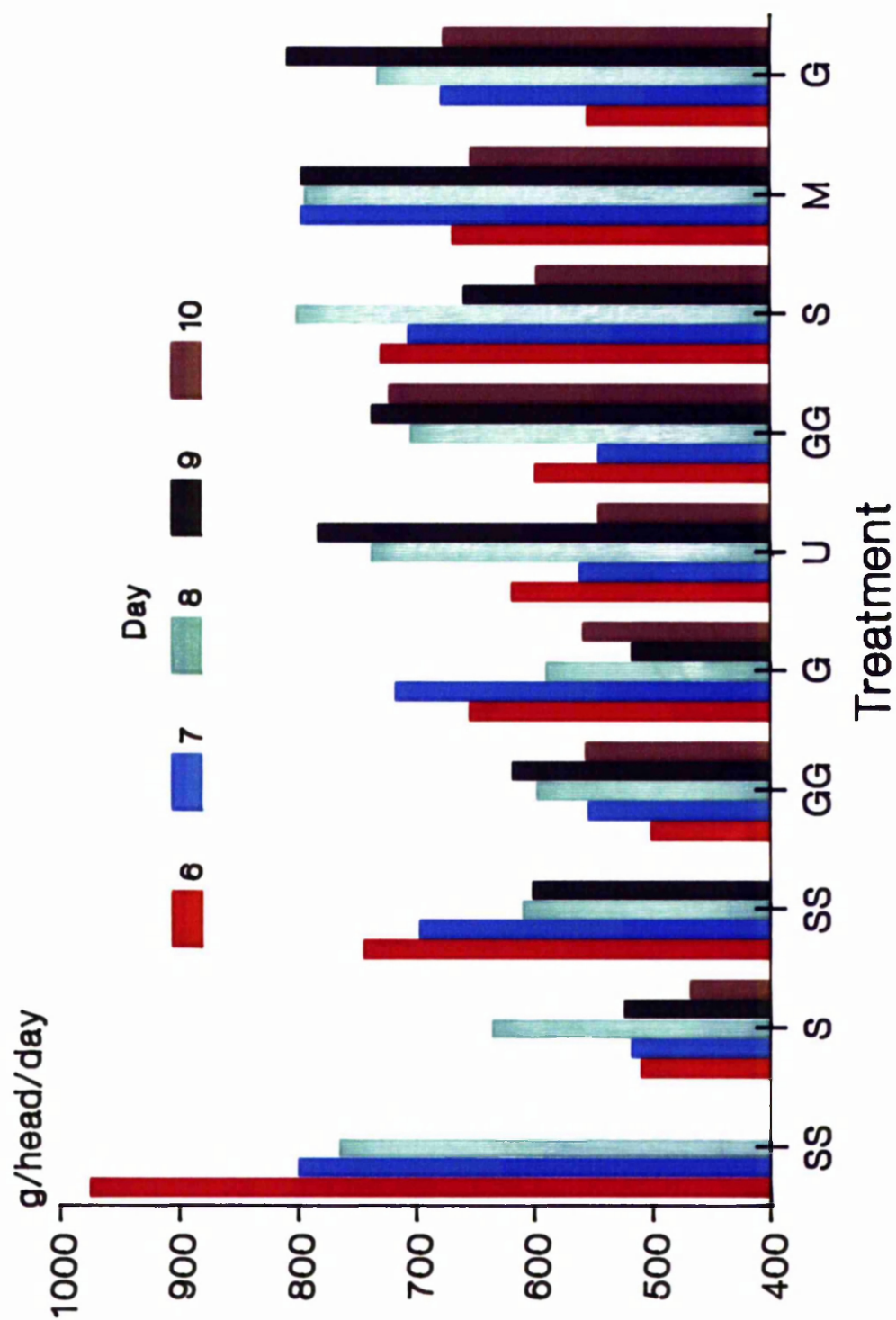
Fermentation end products (water extract analysis) g/kg.

TREATMENT	DM	Ethanol		Acetic		Propionic	
		Fresh	Dry	Fresh	Dry	Fresh	Dry
Graintona 100%	561	3.62	6.45	1.05	1.87	.01	.02
Graintona 50%	548	6.45	11.78	2.74	5.01	.12	.22
Sylade 100%	550	10.55	19.18	.74	1.34	.01	.02
Sylade 50%	543	10.65	19.61	2.55	4.70	.07	.14
Molasses	542	12.68	23.39	4.09	7.54	.02	.03
Untreated	541	5.52	10.21	4.17	7.70	.03	.05
Twice molasses	524	16.66	31.79	6.01	11.46	.03	.06
Add - Safe	540	7.75	14.35	4.24	7.85	.39	.72
Twice Add-Safe	553	1.84	3.32	3.32	6.00	.95	1.72
Molasses + Innoculant	525	16.97	32.31	4.41	8.41	.04	.07
Twice molasses +Innoculant	528	17.01	32.22	7.73	14.64	.05	.10
Innoculant	530	9.57	18.05	3.74	7.07	.02	.04
Amylase	525	11.68	22.26	5.10	9.72	.03	.06
Fresh, frozen	565	2.66	4.71	3.83	6.77	.01	.02

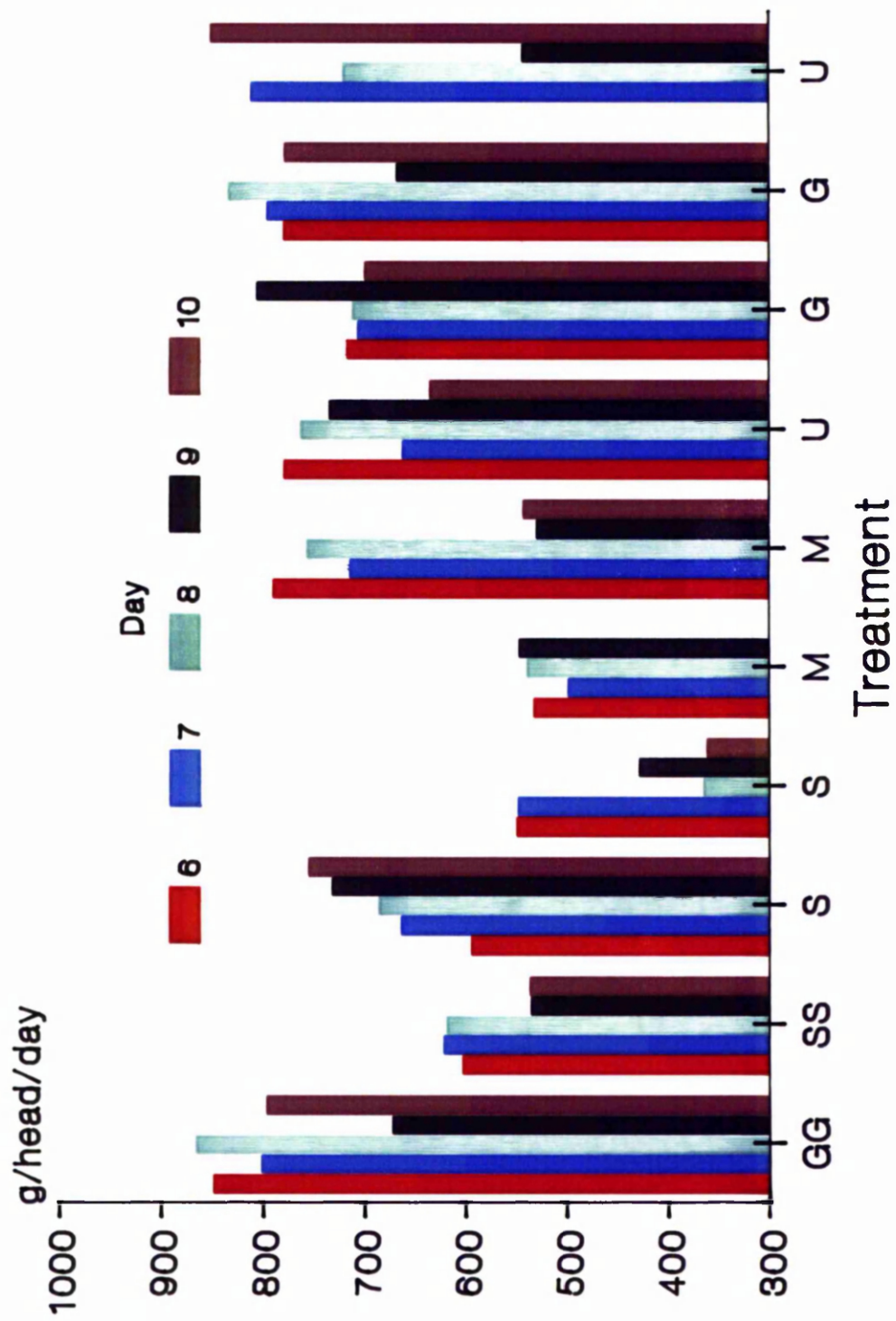
TREATMENT	DM	Isobutyric		Butyric		Lactic	
		Fresh	Dry	Fresh	Dry	Fresh	Dry
Graintona 100%	561	1.68	2.99	.03	.05	.33	.59
Graintona 50%	548	.54	.99	.03	.05	5.83	10.64
Sylade 100%	550	.03	.05	.03	.05	.91	1.65
Sylade 50%	543	.00	.00	.02	.04	4.74	8.74
Molasses	542	.00	.00	.03	.06	9.27	17.10
Untreated	541	.00	.00	.01	.01	8.53	15.77
Twice molasses	524	.00	.00	.01	.01	9.12	17.41
Add - Safe	540	.02	.04	.01	.02	5.86	10.85
Twice Add-Safe	553	.02	.04	.01	.02	3.38	6.11
Molasses + Innoculant	525	.00	.00	.00	.00	7.20	13.71
Twice molasses +Innoculant	528	.00	.00	.01	.02	10.01	18.96
Innoculant	530	.00	.00	.00	.00	6.75	12.74
Amylase	525	.00	.00	.00	.00	8.13	15.48
Fresh, frozen	565	.02	.03	.00	.00	3.27	5.79

APPENDIX E.

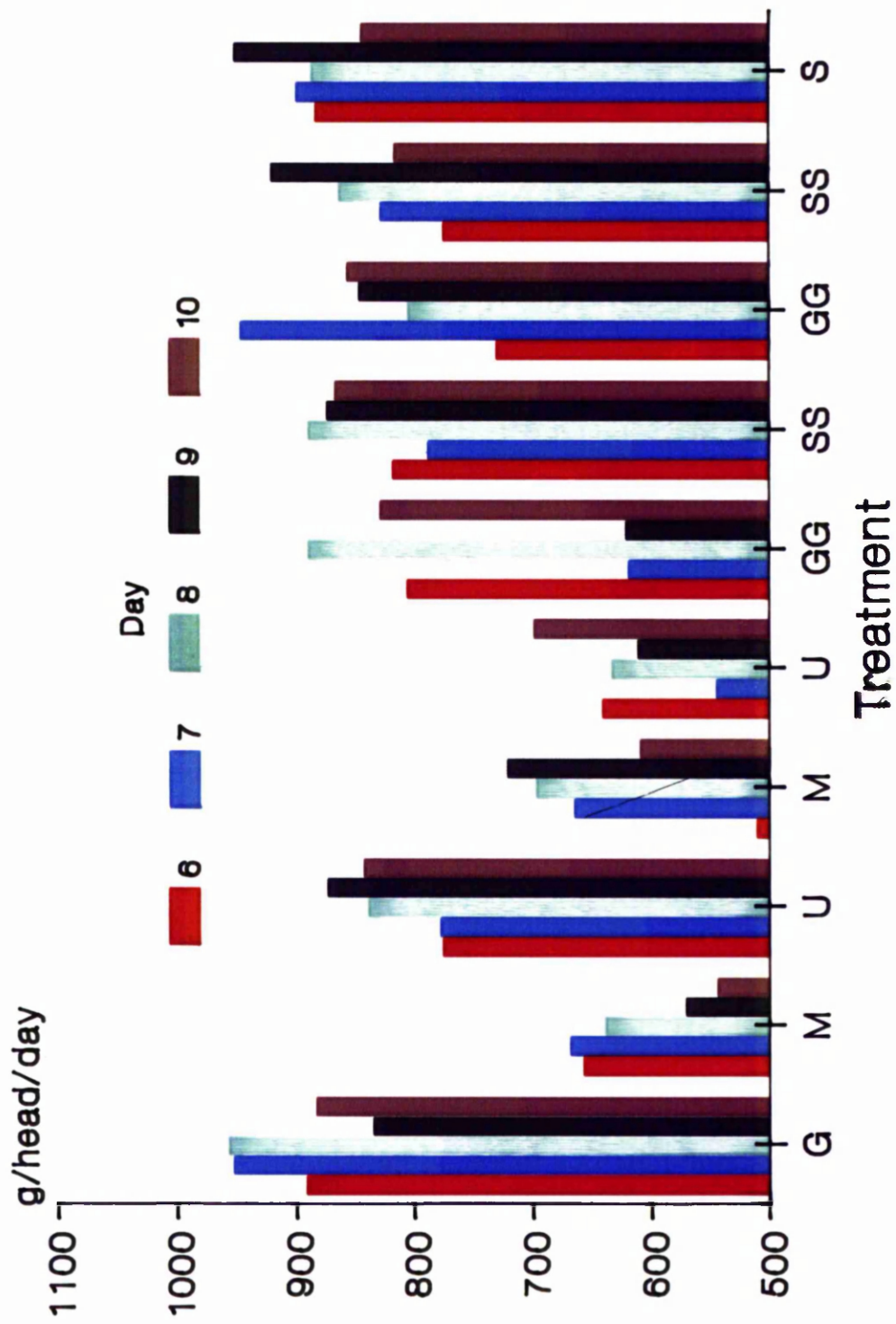
APPENDIX E1 Hay dry matter intake, Period 1, Individual sheep.



APPENDIX E2 Hay dry matter intake, Period 2, Individual sheep.



APPENDIX E3 Hay dry matter intake. Period 3. Individual sheep.



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